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Novel ingredients and emerging processing technologies in the development of clean label, phosphate-reduced meat products

Thesis presented by

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For the degree of

Doctor of Philosophy – Food Science and Technology

Under the supervision of

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Declaration

I, Karthikeyan Palanisamy Thangavelu, hereby declare that this submitted thesis is my work and effort and has not been submitted for another degree at either the National University Ireland, Cork, or elsewhere. All the external references and other sources of information have been used, and they have been acknowledged. I have read and understood the regulations of University College Cork concerning plagiarism and intellectual property.



Karthikeyan Palanisamy Thangavelu

Abstract

Phosphate additives are used in numerous processed foods as stabilisers and emulsifiers. They are present in up to 65% of processed meat products. However, consumer preferences for more natural and less processed foods have resulted in clean-label growth trends, meaning shorter ingredient declarations avoiding ingredients unfamiliar to the consumer. Due to the unique characteristics of phosphates, their removal, while maintaining product quality, is challenging. As an initial study (Chapter 2), a survey assessing the Irish consumer's (n=548) intention to purchase phosphate-reduced processed meat products employing the Theory of Planned Behaviour (TPB) was conducted. Results showed that Irish consumers positively intended to purchase phosphate-reduced processed meat products if widely available. Based on survey findings, this thesis research explored the possibilities of developing high-quality, phosphate-replaced/reduced processed meat products employing natural ingredients and additionally employing novel processing technologies. This objective was achieved by carrying out two important processes: modification of potential phosphate-replacers and meat structure modification using novel processing technologies and accomplished by carrying out four experimental studies. The first experimental study (Chapter 3) examined the phosphate-replacing ability of two dietary fibre-rich food co-product ingredients, namely; apple pomace (AP) and coffee silverskin (CSS), in Irish breakfast sausage formulations. A specialised response surface methodology developed using Mixture design software created 18 sausage formulations containing different sodium tripolyphosphate (STPP), AP and CSS concentrations. Results showed that the addition of ingredients improved WHC ($P<0.001$) and decreased the cook loss ($P<0.001$), hardness ($P<0.001$), chewiness ($P<0.001$) and day 9 TBARS ($P<0.018$) values. Software analysis of results predicted three optimised phosphate-reduced sausage formulations based on the desirability response method. Following chapter 3, efforts were made to enhance the functional properties of AP and CSS using Power Ultrasound (US) technology Chapter 4). This investigated the effect of US treatment of 20 kHz, 250 W at different time intervals of 15 and 30 minutes on the techno-functional properties of AP and CSS, such as water and oil absorption capacity (WAC and OAC), particle size, viscosity, colour and total fibre content. Results indicated that

US treatment on ingredients for 15 and 30 minutes improved WAC and OAC of both ingredients (Significant only for AP). Observation showed that functionality improved with increased treatment duration. This demonstrated that US treatment can create beneficially-functional properties in food co-products or ingredients, such as with AP and CSS powders. Combining Chapters 3 and 4, a comparative study (Chapter 5) was conducted employing US-treated (30 minutes) ingredients in the three optimised phosphate-reduced sausage formulations obtained from the mixture design software. Results showed that the addition of US-treated AP and CSS to all the formulations increased WHC ($P<0.05$), emulsion stability (TEF %) ($P<0.05$), and decreased cook loss ($P<0.05$), demonstrating that the addition of US-treated AP and CSS can improve phosphate-reduced sausage quality. Finally, in Chapter 6, a strategy of using High-Pressure Processing (HPP) technology to alter meat functionality was employed for further sausage quality improvement. Study results showed that HPP (150 MPa for 5 mins) produced positive changes in the water holding capacity (WHC) ($P<0.05$) and cook loss ($P<0.05$) of sausage formulations when compared to non-HPP treated equivalents. In conclusion, there is potential to manufacture sausages with reduced phosphate concentration using novel processing technologies and clean label ingredients, such as those used in this study. Overall, the research presented showed the wide application scope that exists for non-thermal novel processing technology. With further optimisation, these ingredients and the processing technologies employed could be used to produce a wide range of healthier and higher-quality processed meat products.

Abbreviations

ADI - Acceptable Daily Intake

ANOVA- Analysis of Variance

AOAC - Association of Official Analytical Chemists

AP - Apple Pomace

APFD - Apple Pomace Freeze Dried

CKD - Chronic Kidney Disorder

CSO - Central Statistics Office

CSS - Coffee Silver Skin

CSSFD - Coffee Silver Skin Freeze Dried

EFSA - European Food Safety Authority

EU - European Union

FAO - Food and Agriculture Organisation

FDA - Food and Drug Administration

HPP - High-Pressure Processing

IDF - Insoluble Dietary Fibre

LF-NMR- Low Field Nuclear Magnetic Resonance

MDA - Malondialdehyde

OAC - Oil Absorption Capacity

OHC - Oil Holding Capacity

P - Phosphorus

PBC - Perceived Behavioural Control

PEF - Pulsed Electric Field

ROI - Rest of Ireland

RSM - Response Surface Methodology

SAPP - Sodium Acid Pyrophosphate

SDF - Soluble Dietary Fibre

SHMP - Sodium Hexametaphosphate

STPP - Sodium Tripolyphosphate

SW - Shockwave

TDF - Total Dietary Fibre

TEF - Total Expressible Fluid

TPA - Texture Profile Analysis

TPB - Theory of Planned Behaviour

TSPP - Tetrasodium Pyrophosphate

UAE - Ultrasound-Assisted Extraction

US - Power Ultrasound

WAC - Water Absorption Capacity

WBC - Water Binding Capacity

WHC - Water Holding Capacity

WHO - World Health Organisation

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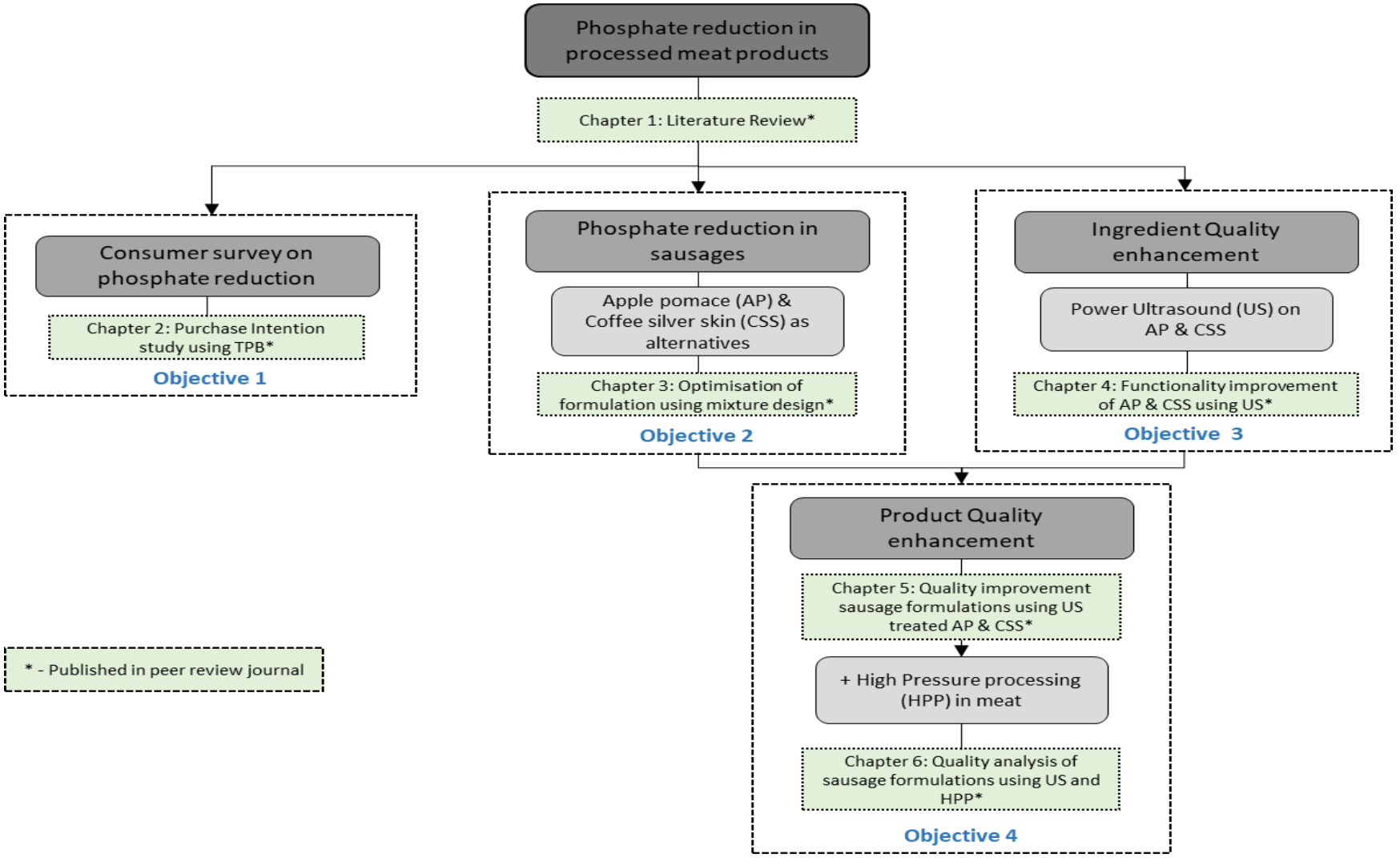
List of Publications emerging from this thesis

1. **Thangavelu, K. P.,** Kerry, J. P., Tiwari, B. K., & McDonnell, C. K. (2019). Novel processing technologies and ingredient strategies for the reduction of phosphate additives in processed meat. *Trends in food science & technology, 94*, 43-53. doi: 10.1016/j.tifs.2019.10.001
2. **Thangavelu, K. P.,** Hyland, J. J., Henchion, M., Kerry, J. P., & Álvarez, C. (2022). Consumer intention towards the phosphate-reduced processed meat products using the extended theory of planned behaviour. *Meat Science*, *193*, 108947. doi: 10.1016/j.meatsci.2022.108947
3. **Thangavelu, K. P**., Tiwari, B. K., Kerry, J. P., McDonnell, C. K., & Álvarez, C. (2022). Phosphate replacing potential of apple pomace and coffee silver skin in Irish breakfast sausage using a mixture design approach. *Meat Science, 185*, 108722. doi: 10.1016/j.meatsci.2021.108722
4. **Thangavelu, K. P.**, Tiwari, B. K., Kerry, J. P., & Álvarez, C. (2022). A comprehensive study on the characterisation properties of power ultrasound-treated apple pomace powder and coffee silverskin powder. *European Food Research and Technology,* 1-11. doi: 10.1007/s00217-022-04017-8
5. **Thangavelu, K. P.,** Tiwari, B. K., Kerry, J. P., & Álvarez, C. A comparative study on the effect of ultrasound treated apple pomace and coffee silver skin powders as phosphate replacers in Irish breakfast sausage formulations. *Foods, 11 (18)*, 2763. doi: 10.3390/foods11182763
6. **Thangavelu, K. P.,** Tiwari, B. K., Kerry, J. P., & Álvarez, C. Effect of high-pressure processing in improving the quality of phosphate reduced Irish breakfast sausage replaced with ultrasound treated phosphate alternatives. *Meat Science, 194,* 108981. doi: 10.1016/j.meatsci.2022.108981

List of Presentations at International conferences

1. **Thangavelu, K. P.,** Tiwari, B. K., Kerry, J. P., & McDonnell, C. K. (2018). The potential of sonicated apple pomace as a phosphate replacer in breakfast sausages. Institute of Food Science and Technology of Ireland (IFSTI), 47th Annual Food Science and Technology Conference, Cork, Ireland (6-7 December 2018).
2. **Thangavelu, K. P.,** Tiwari, B. K., Kerry, J. P., McDonnell, C. K., & Álvarez, C. (2019). Phosphate reducing the potential of apple pomace and coffee silver skin in Irish breakfast sausage using a mixture design approach. ICoMST, 65th International Congress of Meat Science and Technology, Potsdam, Germany (4-9 August 2019).
3. **Thangavelu, K. P.**, Tiwari, B. K., Kerry, J. P., McDonnell, C. K., & Álvarez, C. (2020). Study of changes in the physicochemical properties of ultrasound treated two food by-products apple pomace and coffee silver skin powder. Institute of Food Science and Technology of Ireland (IFSTI), 49th Annual Food Science and Technology Conference, Dublin, Ireland (15 December 2020).
4. **Thangavelu, K. P.**, Tiwari, B. K., Kerry J. P., & Álvarez, C. (2021). Effect of ultrasound treated food ingredients as phosphate replacers in Irish breakfast sausages. ICoMST, 67th International Congress of Meat Science and Technology, Kraków, Poland (23-27 August 2021).
5. **Thangavelu, K. P.**, Tiwari, B. K., Kerry J. P., & Álvarez, C. (2022). Investigating the effect of high-pressure technology on the quality of phosphate-reduced sausage formulations with ultrasound-treated ingredients as phosphate alternatives. ICoMST, 68th International Congress if Meat Science and Technology, Kobe, Japan (22-23 August 2022).
6. **Thangavelu, K. P.**, Hyland, J.J., Henchion, M., Kerry, J. P., & Álvarez, C. (2022). Measuring the purchase intention of Irish consumers towards phosphate-reduced processed meat products using extended Theory of Planned Behaviour. ICoMST, 68th International Congress if Meat Science and Technology, Kobe, Japan (22-23 August 2022).

Schematic Representation of Thesis Workflow Structure



Chapter 1. Literature Review

Overview

This chapter discusses the interaction between novel technologies and natural ingredients in replacing the phosphate additives added to processed meat products. The review study examined the changes produced in the physicochemical properties of processed meat products by applying these technologies and alternate ingredients. Limitations and challenges associated with this approach were also outlined. The following paper has been published from this chapter in a peer-reviewed journal:

- **Thangavelu, K. P.,** Kerry, J. P., Tiwari, B. K., & McDonnell, C. K. (2019). Novel processing technologies and ingredient strategies for the reduction of phosphate additives in processed meat. *Trends in food science & technology, 94*, 43-53.

1.1 Introduction

Phosphates are essential for human health as they are required for the growth, maintenance, and repair of cells and tissues, signalling, energy transfer and other important functions. They are involved in many metabolic pathways and are naturally found in the form of organic esters in foods like eggs, meat, potatoes, and cereals. In general, the acceptable daily intake (ADI) of phosphorus (P) for a healthy adult is 40 mg/kg body weight per day (Younes et al., 2019). Commonly, higher quantities are consumed, but the kidneys readily excrete excess phosphate. However, individuals with poor kidney function, such as those with chronic kidney diseases (CKD), must closely monitor their dietary intake of phosphate to avoid an occurrence of hyperphosphatemia (Calvo & Uribarri, 2013; Kalantar-Zadeh et al., 2010; Ritz, Hahn, Ketteler, Kuhlmann, & Mann, 2012). This is particularly important with the increased use of inorganic P-containing additives, such as phosphate (P_2O_5), in processed foods (Winger, Uribarri & Lloyd, 2012).

Inorganic phosphates are generally considered safe (GRAS) by the United States Food and Drug Administration (FDA). They are used as an effective food additive in many processed food products such as meat, ham, sausages, cheese, canned fish, beverages and baked products. Phosphate addition in the US is regulated by FDA regulations that control the maximum usage levels in food products (Dykes, Coorey, Ravensdale, & Sarjit, 2019). Since 1990, due to increased consumption of processed foods, P intake has doubled from 500 mg/day to 1000 mg/day in the American diet (Kalantar-Zadeh et al., 2010). Studies by Leon, Sullivan, & Sehgal (2013) showed that processed food contributed to an extra 700-800 mg of P daily intake. Almost 44% of best-selling groceries in America contain phosphate additives.

The increase in the use of phosphates in processed foods may be due to their unique characteristics, which often improve product quality. Phosphates serve as buffers, sequestrants, acidulants, bases, gel accelerants, dispersants, precipitants, and ion-exchange agents. In the EU, phosphates are classified in the Additive Directive (Regulation EC 1333/2008) as belonging to various functional classes such as emulsifiers, stabilisers, sequestrants, and thickeners. Their use is permitted in several processed food categories. Phosphates serve several functions in processed meat,

such as stabilising pH, increasing water holding capacity (WHC), decreasing cooking loss, improving texture and sensory qualities and more (Dykes et al., 2019). As per the EU legislation on food additives, the maximum allowed concentration of phosphates in processed meat products is 5000 mg/kg expressed as P_2O_5 content (Regulation (EC) 1333/2008, 2008).

Due to the increased consumption of processed foods, the safety of phosphate additive consumption was recently re-evaluated. The European Food Safety Authority (EFSA) scientists have estimated that P's ADI from food is protective for healthy individuals and within the safe level previously set by EFSA. However, dietary exposure to phosphorus might exceed the ADI in infants, children, and adolescents with a high phosphate diet (Younes et al., 2019). Also, in recent times, there has been a general shift towards alternative ingredients in food products with the emergence of consumer trends such as health concerns, sustainability, and convenience (Asioli et al., 2017). For sustainable processing, alternatives to synthetic phosphates (e.g. valorisation of functional ingredients from coproducts and waste streams) could offer an opportunity for a sustainable circular economy in the food sector. Consumer preference toward natural and less processed food has resulted in the growth of the clean-label trend. The term clean label first appeared during the 1980s, meaning food products without any E-number additives on the food label. The E numbers identify the food additives permitted for use within the European Union by the European Food Safety Authority (Asioli et al., 2017). Although, with the growing trend, the term 'clean label' is difficult to define (Asioli et al., 2017). Ingredion (2004) suggests clean labelling in Europe as the products positioned as natural, organic and/or free from additives/ preservatives, which is very similar to the approach of 'natural labelling' by the FDA, referring to products containing no artificial or synthetic additives.

The clean label trend has become prominent as many new food products contain fewer inorganic additives (Asioli et al., 2017). However, consumers must understand that a functional ingredient, such as P_2O_5, is only added to the EU Additive Directive by complying with the conditions set out in Regulation 1333/2008. In addition, the Additive Directive sets safe limits on the acceptable levels of these ingredients in food products. Nonetheless, there remains an interest in replacing the functional

properties of phosphates with clean-label alternatives. In that sense, the chosen component must have techno-functionality. In the EU, food additives are substances not generally consumed as the food itself but are added to food intentionally for a technological purpose (Regulation (EC) 1333/2008). With uncertainty in the precise definition of natural antimicrobials, colourants, sweeteners or antioxidants (Carocho et al., 2015), it is more challenging to define natural techno-functional ingredients when also other aspects like GM-free and allergens are considered. In that sense, there is difficulty in truly classifying 'clean label' ingredients. Henceforth, all possible alternative ingredients, irrespective of clean label status, have been discussed in this review in the later sections.

Various attempts have been made to replace phosphates in meat with suitable ingredients like starches, proteins, seaweeds, hydrocolloids and fibres (Younis & Ahmad, 2015; Resconi, Keenan, Barahona, et al., 2016a). However, the complete replacement of phosphate in meat with alternative ingredients may negatively affect appearance, texture and other major product characteristics. For example, rice starch as a phosphate replacer in whole muscle-cooked hams involves meat's formation and sensory qualities ((Long, Gál, & Buňka, 2011; Resconi, Keenan, Barahona, et al., 2016a). Similar results were obtained when the amount of phosphate added to meat was reduced without adding any functional ingredients (Glorieux, Goemaere, Steen, & Fraeye, 2017). Studies have shown that alternative technologies can effectively enhance the quality of meat processed with added alternative ingredients. Among the technologies, high-pressure processing (HPP) proved to improve the functionality of meat products by altering the meat structure. HPP application in meat products can modify the spatial protein structure resulting in the solubilisation of myofibrillar proteins. This can reduce the quantities of salts and phosphates required in processed meat (Tamm, Bolumar, Bajovic, & Toepfl, 2016). For example, the reduced-salt cooked ham was produced without changes in WHC and texture using a salt replacer (KCl) and HPP at 100 MPa (Tamm et al., 2016). Similarly, power ultrasound (US) technology has been widely used to assist in effective ingredient distribution and diffusion within food matrices. For example, the

US has been shown to accelerate the diffusion of salt (McDonnell et al., 2017) and salt replacers in pork tissue (Ojha, Keenan, Bright, Kerry, & Tiwari, 2016).

In line with the trend for healthier processed meats, comprehensive reviews exist on strategies for sodium reduction (Inguglia, Zhang, Tiwari, Kerry, & Burgess, 2017) and nitrite reduction (Bedale, Sindelar, & Milkowski, 2016) in processed meats. However, there is a lack of research on phosphate reduction. This review aims to discuss the potential of alternative ingredients and novel processing technologies to reduce phosphates in processed meats.

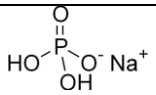
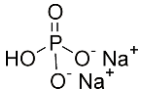
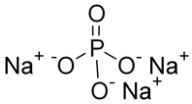
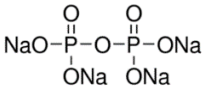
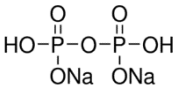
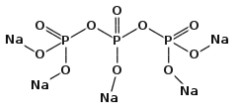
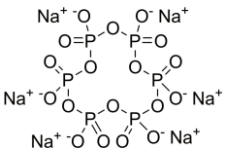
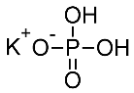
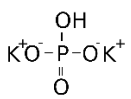
1.2 Phosphates in Processed Meat

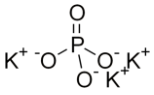
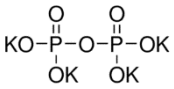
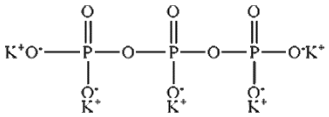
Phosphates in processed meat products are the salts of phosphoric acids containing sodium or potassium's positively charged metal ions. Depending on the country, various legislations exist on phosphate additives used in foods, and further information on this can be found in Dykes et al. (2019). As per European legislation, food-grade phosphates are not permitted in fresh meat; however, they can be added in a limited concentration to meat preparations and meat products (Regulation (EC) 853/2004). According to the Food and Agriculture Organization (FAO) and World Health Organisation (WHO) food standards, the maximum permitted level of phosphates in finished products, whole pieces or cuts, and processed comminuted meat products is approximately 5041 mg/kg expressed in P_2O_5 (Codex Standard 192, 1995, Balestra & Petracci, 2019).

1.3 Types of Phosphates Used in Meat

Several molecular forms of phosphate (P_2O_5) exist, selected depending on their required function in the food matrix. Phosphates are classified according to the number of phosphorus atoms sharing oxygen atoms (Lampila & Godber, 2002). They are ortho- or monophosphates with one phosphate molecule, di- or pyrophosphates with two phosphate molecules, triphosphates with three phosphate molecules and polyphosphates with more than three phosphate molecules. The molecular structures of phosphates are ring or metaphosphates, chain /linear phosphates or ultra /branched phosphate structures with a combination of round and linear phosphates (Table 1-1).

Table 1-1: List of phosphates used in meat products with corresponding P_2O_5 content adapted from Long et al. (2011) and Lampila and McMillin (2017). *E numbers – stands for the codes for the food additives permitted to use within the European Union by the European Food Safety Authority. Roman numerals in the E numbers denote the different types of phosphate with the same cationic group. For example, E339 (i), (ii), and (iii) represent the different types of sodium phosphate groups, while E450 (i) and (iii) denote the different sodium pyrophosphates.

Common names	Chemical structure	pH (1% solution)	P_2O_5 content (%)	E* number
Monosodium phosphate		4.4-4.8	59.2	E339(i)
Disodium phosphate		8.6-9.4	50.0	E339(ii)
Trisodium phosphate		11.9-12.5	43.3	E339(iii)
Tetrasodium pyrophosphate		9.9-10.7	53.4	E450(iii)
Sodium acid pyrophosphate		4.0-4.4	64	E450(i)
Sodium tripolyphosphate or Pentasodium phosphate		9.5-10.2	57.9	E451(i)
Sodium hexametaphosphate		6.3- 7.3	69.6	E452(i)
Potassium monophosphate		4.4-4.8	52.1	E340(i)
Dipotassium phosphate		8.6-9.4	40.8	E340(ii)

Tripotassium phosphate		11.9-12.5	33.4	E340(iii)
Tetrapotassium pyrophosphate		10.0-10.5	43.0	E450(v)
Potassium tripolyphosphate		9.5-10.2	47.5	E451(ii)

In many countries, only linear phosphates were permitted in processed meats (Long et al., 2011). The commonly used Graham's salt (sodium hexametaphosphate) is a linear phosphate with a P_2O_5 content of about 60-70% (Feiner, 2006; Lampila & Godber, 2002). Sodium tripolyphosphate (STPP) is commonly mixed with sodium hexametaphosphate (SHMP), tetrasodium pyrophosphate (TSPP) or sodium acid pyrophosphate (SAPP) for use in meat products like ham, bacon, frankfurters, bologna, precooked breakfast sausages, delicatessen meats, breaded chicken products and injected poultry pieces (Lampila, 2013). Different phosphates are used for other meat products based on production and formulation, as Long et al. (2011) explained. For example, long-chain polyphosphates with better solubility are used to prepare brine solutions for ham. In contrast, short-chained phosphates are used for emulsified products like sausages, where the added phosphates act on the protein instantly (Feiner, 2006).

1.4 Functionality of phosphates in meat

Phosphates have various functionalities such as buffering, water-binding, emulsification, colour stability, oxidation inhibition, antibacterial activity, and protein dispersion properties (Figure 1-1). Still, they are most commonly used in meat products for their emulsifying and stabilising capabilities, which primarily affect the WHC (Nguyen, Gal, & Bunka, 2011).

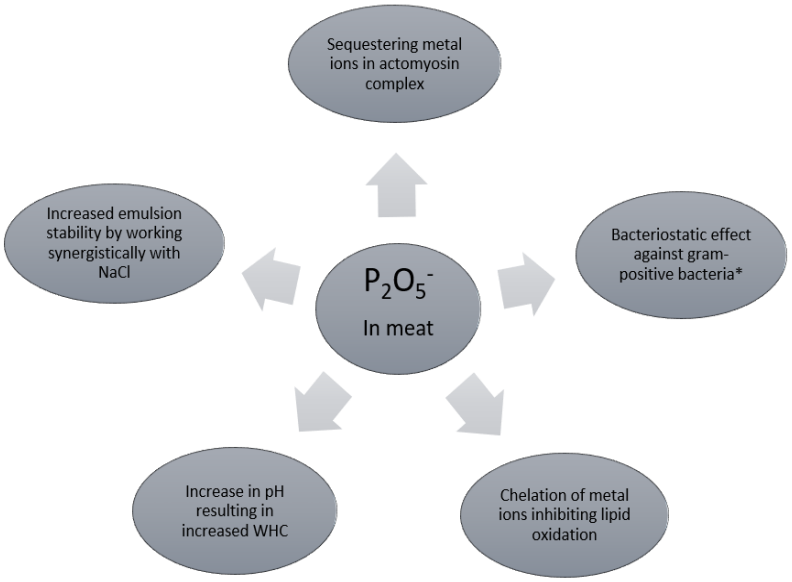


Figure 1-1: Some important functionalities of phosphates in meat products.

*However, the quantities used in meat are not high enough to have a significant bacteriostatic effect.

Water holding capacity (WHC) is the ability of meat products to retain their inherent water when external pressure or force is exerted upon them and during storage, thereby affecting weight and juiciness (Gyawali & Ibrahim, 2016). In general practice, salting is one of the oldest techniques for preserving meat and may increase WHC depending on the concentration added (Feiner, 2006). Due to several underlying mechanisms, phosphate additives may work synergistically with NaCl to decrease water losses. Significantly, phosphates affect the intrinsic pH of meat by moving from the isoelectric point (pI). For this reason, most phosphates used in meat products are alkaline (Long et al., 2011), except sodium acid pyrophosphate, which is acidic and is used for various functions, such as accelerated cured colour formation by nitrite (Lampila, 2013). The increase in pH results in the increased electrostatic repulsion between the proteins allowing for water entrapment (Puolanne, Ruusunen, & Vainionpaa, 2001). This, in turn, results in swelling of the muscle fibres and activation of proteins. This swollen and active protein traps and immobilises water added to the

meat. Hence, the WHC is increased, which is especially true in the case of polyphosphates like SHMP and STPP (Glorieux et al., 2017).

Phosphates also increase the WHC of meats by sequestering the metal ions such as Ca^{2+}, Mg^{2+}, Fe^{2+} and Fe^{3+} present in the actomyosin complex (Long et al., 2011). When added, phosphates can bind with ions in the actomyosin complex formed during rigor mortis. Dissociation of actomyosin into actin and myosin increases the solubilisation of meat proteins through depolymerisation of thick and thin filaments, which leads to increased WHC, emulsifying and gelling properties (Glorieux et al., 2017; Puolanne & Halonen, 2010). This can also positively impact the textural characteristics of meat products.

Phosphates also work synergistically with NaCl for improved product quality. Shults, Russell, & Wierbicki (1972) first demonstrated the synergetic relationship between salts and phosphates. For centuries, sodium chloride (NaCl) salt was used in meat products for preserving and increasing WHC. This is mainly due to the positive effect of NaCl on the solubility of myofibrillar proteins. The Cl^- ions induce electrostatic repulsion between the meat proteins and meat swelling. Generally, a minimum concentration of 0.6M of NaCl is required to extract myofibrillar proteins from the muscle. Still, NaCl can be effectively reduced by adding phosphates (5000 mg/kg) to meat product formulations. Thus, phosphate facilitating actomyosin dissociation makes myosin more easily solubilised by NaCl, immobilising large amounts of added water. Studies by Schwartz & Mandigo (1976) proved the synergic effect of salt (0.75%) and STPP (0.125%) on restructured pork in improving the WHC, eating texture, aroma, flavour, cooking loss, and juiciness upon storage at -23 ^oC for four weeks when compared to salt alone. Later, Knight & Parsons (1988) were one of the first to describe the myofibril's structural changes following NaCl and polyphosphate treatments. Numerous studies were carried out which demonstrate this synergy and the WHC properties of added phosphates on meat products (Erdogdu, Erdogdu, & Ekiz, 2007; Puolanne et al., 2001; Sen, Naveena, Muthukumar, Babji, & Murthy, 2005).

Phosphates and NaCl also improve meat products' emulsion stability by allowing myosin to form a sticky protein substance upon mixing, known as the exudate that forms a gel upon heating (Lampila, 2013). This helps bind the pieces of meat in the production of reformed products. This water-fat-protein emulsion matrix development is also critical in frankfurter and bologna production. The application of phosphates and NaCl in meat formulations results in myosin solubilisation, orienting its hydrophobic tail around a fat droplet and binding its hydrophilic end with water (Lampila & McMillin, 2017). When heated, myosin undergoes several structural changes like oxidation of sulfhydryl (SH) groups to form filamentous gel structure at 20-50 ^{o}C. Further, at 50 -70 ^{o}C, the second stage of gelation occurs where myosin tails undergo cross-linking and strengthen the gel structure and emulsion stability, increasing the WHC and reducing the cooking loss. However, the temperature ranges for the structural transitions are dependent on several factors within the protein system (e.g., species, pH, ionic strength, ingredients) (Chen et al., 2018). To prove the emulsifying property, Anjaneyulu, Sharma, & Kondaiah (1990) studied the effect of blends of phosphates (65% TSPP, 17.5% STPP, and 17.5% SAPP) on buffalo meat patties with added 2% NaCl. The results showed improved emulsifying capacity and emulsion stability with increased WHC.

The chelating properties of phosphates also provide some anti-oxidative ability. Lipid oxidation may be inhibited by phosphates chelating with metal ions that otherwise could catalyse the oxidation of proteins like haemoglobin and lipid-like phosphor-lipids. Therefore, their inclusion in products could prevent colour degradation and the generation of rancid off-flavours (Dykes et al., 2019; Feiner, 2006; Long et al., 2011). Fernandez-Lopez, Sayas-Barbera, Perez-Alvarez, & Aranda-Catala (2004) showed sodium tripolyphosphate (0, 0.15 or 0.30%) in pork meat resulted in reduced lightness and stabilised the percentage of oxymyoglobin. However, no effect was seen on redness, yellowness, chroma and hue saturation of meat colour. Studies by Baublits, Pohlman, Brown, & Johnson (2005, 2006); Fernandez-Lopez, Sayas-Barbera, Perez-Alvarez, & Aranda-Catala, (2004) help in understanding the functions of phosphate mixtures on the colour properties of meat products.

Phosphates can also act as a preservative with a slight bacteriostatic effect against some gram-positive bacteria. However, meat products are less significant as a greater concentration of phosphates, or additional preservatives are required for effective antibacterial activity (Feiner, 2006; Long et al., 2011).

1.5 Strategies to reduce phosphates in meat products

Consumers' awareness of food additives and their interests in clean-label food products has led to a need to reduce and/or remove phosphates and often replace them with various functional ingredients that can serve as fillers, binders, emulsifiers, and stabilisers. This can be achieved by product reformulation and/or process modification. Strategies for replacing phosphates in meat with suitable phosphate replacers and novel processing technologies are summarised in Figure 1-2. The identified technologies with potential are HPP and US since other technologies such as ozone, pulsed UV-light and plasma may have surface decontamination potential (Troy et al., 2016) and therefore do not fall within the scope of this review. Pulsed electric fields and shockwaves are two emerging technologies for meat tenderisation, with limited research on their potential application in processed meats. While this information is included, emphasis is placed on the discussion of US and HPP that show more potential in applying phosphate-free meat products. Thus, this review will discuss the possible ingredient and technology approaches for phosphate reduction in meat products concerning specific quality characteristics, including water binding, emulsion stability, sensory, texture, colour, and oxidative status.

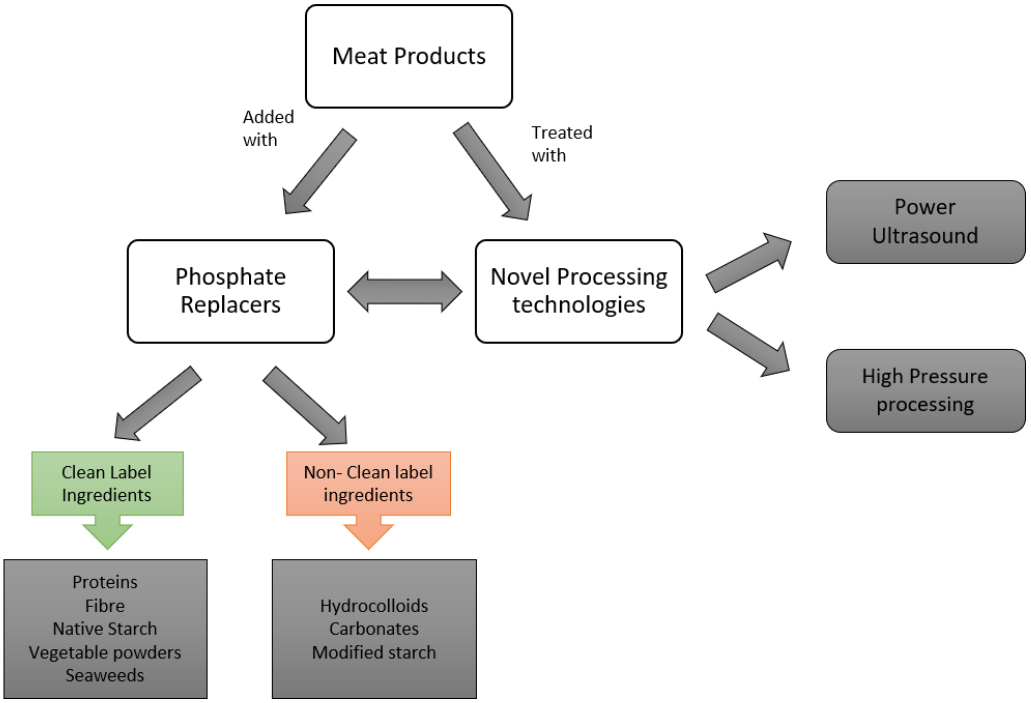


Figure 1-2: Phosphate replacing strategy in meat products using phosphate replacers and novel processing technology

1.6 Ingredient strategies for phosphate reduction in processed meat

Various alternative functional ingredients for phosphates include native and modified starches, proteins, dietary fibres, hydrocolloids, seaweeds, vegetable powders, carbonate salts, and high pH alkaline solutions. These ingredients can offset some quality losses when phosphates are removed or reduced (Resconi, Keenan, Barahona, et al., 2016a; Glorieux et al., 2017). These alternative ingredients can be added in small quantities to replicate some of the functionalities of phosphates in meat products. As discussed earlier, the ingredients, irrespective of their clean label status, are discussed in this section for their ability to replace various techno-functionalities of phosphates such as WHC and cook yield, emulsion stability, and textural and sensory properties. Table 1-2 lists the different ingredients that could be used as phosphate replacers based on their ability to produce specific characteristics in meat products.

Table 1-2: List of different ingredients that could be used as a potential phosphate replacer based on the techno-functionality and the characteristic they impart on different meat products.

Techno - functionality	Ingredients	Meat product	Effects	Reference
Water holding capacity & cook yield	Potato starch and sodium carbonate	Pork loin	Improved cook yield	Prabhu & Husak (2014)
	Rice starch and fructooligosaccharides	Cooked hams	Improved WHC and negative cook yield	Resconi, Keenan, Barahona, et al. (2016a)
	SavorPhos containing citrus fibre	Marinades for rotisserie birds and boneless-skinless breast	Same WHC and cook yield when compared to the control	Casco et al. (2013)
	Pea and carrot fibre	Comminuted meat products	Increased WHC	Petracci et al. (2013)
	Seaweed *H. elongata*	Beef patties	Improved water binding and cooking yield	Cox & Abu-Ghannam (2013)
	Dehydrated beef protein	Brines for beef steaks	Decreased total fluid loss	Lowder et al. (2011)
	Cuttlefish gelatine	Turkey meat sausages	2.5% increase in WHC	Jridi et al. (2015)

Pea protein	Comminuted meat products	Improved WHC and decreased cook and purge loss	Pietrasik & Janz, 2010; Sanjeewa, Wanasundara, Pietrasik, & Shand (2010)
Rye bran, oat bran and barley fibre	Low-fat sausages and meatballs	Oat bran (6%) increased gelling properties and decreased frying loss in sausages, while rye bran (2.1%) improved sensory characteristics	Petersson, Godard, Eliasson, & Tornberg (2014)
Carrageenan	Tumbled meat product	Improved cook yield, WHC	Petracci et al. (2013)
Sugarcane dietary fibre	Low-fat meat batter	Increased water and fat-binding. 2% sugarcane dietary fibre resulted in comparable acceptability to the control	Zhuang, Han, Kang, Wang, Bai, Xu, & Zhou (2016)
Carrageenan	Turkey sausages	Increased WHC	Ayadi et al. (2009)
Sodium bicarbonate	Cooked chicken breast fillets	Increased WHC and texture properties	Mudalal et al. (2014)
Microbial transglutaminase	Pork batter gel	Decreased cooking loss with an increase in transglutaminase concentration	Pietrasik & Li-chan (2002)
Sodium bicarbonate	Marination of broiler breast meat	Higher water retention and improved cook yield	Petracci et al. (2012)

	Sodium bicarbonate	White shrimp	Improved WHC and cook yield	Wachirasiri et al. (2016)
Emulsion stability	Apple pomace powder	Buffalo sausages	Improved emulsion stability and water activity	Younis & Ahmad (2015)
	Apple pomace powders	Reduced-fat chicken sausages	Increased emulsion stability	Choi et al. (2016)
	Carrageenan	Meat emulsion gel	Increased emulsion stability	Paglarini et al. (2018)
	Makgeolli lees fibre	Reduced-fat pork frankfurters	A 10% fat reduction can be achieved, with similar product characteristics, by 2% fibre addition	Choi, Park, Kim, Hwang, Song, Choi, Kim (2013)
	Pig plasma transglutaminase	Low-salt chicken meatballs	Increased gel strength and high emulsion stability	Tseng, Liu, Chen (2000)
	Mushroom powder Agaricusbisporus	Meat emulsion batters	Increase in emulsion stability	Kurt & Genccelep (2018)
Textural and Sensory Quality	Winter mushroom powder	Emulsion type sausages	Inhibited lipid oxidation and better textural properties	Choe et al. (2018)
	Rice starch and potato starch	Reformed ham	Reductions in sensory qualities were observed when phosphate was removed entirely	Resconi, Keenan, Garcia, et al. (2016b)

Soy hull pectin and insoluble fibre	Beef burger patty	Pectin minimised water loss and texture defects	Kim, Miller, Lee, & Kim (2016)
Wheat fibre	Reduced meat and fat burger patty	Up to 3.75 g fibre addition achievable with the same sensory acceptance as the control	Carvalho, Pires, Baldin, Munekata, de Carvalho, Rodrigues, Trindade (2019)
Citrus fibre	Uncured all-pork bologna and oven-roasted turkey breast	Products had similar physical, chemical and sensory characteristics to products with phosphates in them	Powell (2017)
H. elongata	Frankfurters and breakfast sausages	Enhanced hardness and chewiness	Lopez-Lopez, Cofrades, Ruiz-Capillas, & Jimenez-Colmenero (2009)
Seaweeds *L. japonica*	Fat-reduced pork patties	Improved textural and sensory qualities	Choi et al. (2012)
Sodium carbonate and inulin	Restructured poultry steaks	Same sensory and textural qualities	Őztűrk and Serdaroğlu (2017)
Potassium carbonate	Pork meat	Increased sensory quality	LeMaster et al. (2019)
Ammonium hydroxide (1%)	Brine for beef strip loins	Improved display quality	Parsons et al. (2011a)
Alkaline electrolysed water	Pork loin	Negative textural and sensory properties	Rigdon et al. (2017a)

1.6.1 Water binding and emulsifying properties

One of the main techno-functionalities of phosphates in meat products is increasing the WHC and cooking yield (Nguyen, Gal, & Bunka, 2011). Ingredients like starches, proteins, fibres, hydrocolloids, and bicarbonate salts can improve WHC and cook yield when used in meat products (Petracci et al., 2013). Many studies have been made on these ingredients to enhance the WHC of meat products without any added phosphates (Resconi, Keenan, Garcia, et al., 2016b; Prabhu & Husak, 2014; Casco et al., 2013; Sousa et al., 2017). For example, Wachirasiri et al. (2017) investigated the phosphate replacing ability of sodium bicarbonate at low concentrations for frozen white shrimp (*Penaeus vannamei*). The shrimps were treated with sodium bicarbonate ($NaHCO_3$), lysine, and $NaHCO_3$/lysine mixtures at various concentrations and before freezing. Results of thawing yield, cooking yield, colour, and textural values were compared with a control (sodium tri polyphosphates (STPP) treated shrimps). It was concluded that treating shrimps with $NaHCO_3$ /lysine at 1% (w/v) improved the WHC and cooking yield (100.45%, w/w), similar to that of STPP treated samples (101.73%, w/w), proving that $NaHCO_3$ can act as a possible phosphate replacer. In a study by Casco et al. (2013), SavorPhos (a mixture of citrus flour rich in fibre content, all-natural flavourings and less than 2% sodium carbonate) was used as a phosphate replacer in water and oil-based marinades in rotisserie birds and boneless-skinless breast. SavorPhos, when used in water marinade, resulted in equal WHC and cook loss as that of the control phosphate blend, whereas when used in oil marinade, it increased WHC and decreased cook loss. The study of Bertram, Meyer, Wu, Zhou, and Andersen (2008) elucidated the structural changes induced by sodium bicarbonate ($NaHCO_3$), salt (NaCl), and tetrasodium pyrophosphate ($Na_4O_7P_2$) in enhanced pork by use of low-field nuclear magnetic resonance and confocal laser scanning microscopy. It was found that sodium bicarbonate ($NaHCO_3$) resulted in increased solubilisation of proteins and a higher degree of swelling of the myofibril, resulting in increased yield and reduced cooking loss (Bertram et al., 2008).

Similarly, starches have the potential to affect the water-binding properties of meat. In the study made by Genccelep, Saricaoglu, Anil, Agar, and Turhan (2015), both physically and chemically modified starches were used to study the steady-state and

dynamic rheology of meat emulsions. In the study, acid-modified starch (AMS), dextrins modified starch (DMS) and pre gelatinised modified starch (PGS) are compared with native potato starch (NPS). The results concluded that PGS is a good thickener and can be used as a stabiliser for meat emulsions due to their higher water and oil binding capacity, particle size, intrinsic viscosity, and solubility NPS. Thus, there is evidence that starches can be modified to impart specific characteristics in meat products. It should be noted that physically modified starches are modified without enzymatic hydrolysis and chemicals. Therefore, they are declared similarly to native starches while often having more functionality than native starches. They do not have an E-number, making them functional ingredients that fit the clean label trend.

Like WHC, studies have proven the emulsion stabilising property of different ingredients in meat emulsions. Native starches, fibres, seaweeds, vegetable powders, and hydrocolloids can improve emulsion stability in meat batters (Petracci et al., 2013). Studies by Youssef & Barbut (2011) revealed that adding soy protein isolates to lean meat emulsion batters increased moisture retention, emulsion stability, and decreased cook loss. Similarly, Paglarini et al. (2018) studied the influence of carrageenan on WHC of meat emulsion gels at different concentrations of the mixture using Plackett–Burman design. Results revealed that carrageenan addition increased the WHC of the emulsion mixture and improved emulsion stability.

While research has shown that many ingredients can increase the water-binding and emulsification of meat matrices, as shown in Table 1-2, protein solubilisation and muscle binding remain challenging when phosphates are removed. Like phosphate, these ingredients do not act on the actomyosin complex (Prabhu & Husak, 2014). One specific challenge is the binding of pieces to create reformed products; as it is, it is difficult to form a sticky exudate without phosphate, for which transglutaminase could be an option (Feriner, 2006; Lampila, 2013).

1.6.2 Texture and sensory characteristics

Phosphates play a significant role in the textural properties of meat products. Many studies have assessed the effect of potential phosphate replacing ingredients on meat's textural and sensory characteristics. In a study by Cox & Abu-Ghannam (2013), adding seaweed, H. elongata, at different concentrations (0, 10, 20, 30 & 40%) to beef patties improved water-binding, decreased cook losses, increased tenderness and improved sensory properties. Similar results were obtained when the *H. elongata* (5.5%) was incorporated in frankfurters and breakfast sausages, whereby the hardness and chewiness of the products were enhanced upon their addition (Lopez-Lopez, Cofrades, Ruiz-Capillas, & Jimenez-Colmenero, 2009). A recent study by Choe et al. (2018) using winter mushroom powder (*Flammulina velutipes*) as a phosphate replacer in emulsion-type sausages showed that adding 1% of mushroom powder inhibited lipid oxidation and produced better texture characteristics. When added to meat products, bicarbonate salts increase textural quality by improving juiciness and reducing drip loss and shear force.

Though the ingredients have demonstrated some ability to replace phosphates, there are sometimes negative quality attributes associated with phosphate replacement. For example, although there was improved WHC and decreased cooking and purge losses, studies revealed that incorporating pea proteins in meat products produced a negative impact on the textural attribute (Pietrasik & Janz, 2010; Sun & Arntfield, 2012). Studies by Resconi, Keenan, Garcia, et al. (2016b) suggested that a reduction in phosphate content could be made by adding a significant amount of starch to the reformed hams without compromising the quality. However, a reduction in the sensory quality was observed when phosphates were entirely replaced by rice or potato starch. Hence, some ingredients that have demonstrated potential could be optimised with further research, but it remains challenging to replace phosphates due to their multi-functionality in meat products.

1.6.3 Colour and oxidative stability

In principle, phosphates play a small role in controlling lipid oxidation and improving the colour stability of meat products (Choe et al., 2018). While most research has been conducted with emphasis on other quality parameters, some research has been

conducted on the effect of phosphate alternatives on colour and oxidative stability. A study by Choe et al. (2018) showed no significant colour difference in the emulsion-type sausages when added to winter mushroom powder. In contrast, in the study by Choi et al. (2016), adding apple pomace fibre to fat reduced chicken sausages and affected the product's colour. Thus, the colour of the meat products may vary according to the type of ingredients used as some ingredients may have a naturally darker colour than the meat or phosphates and thereby contribute to the colour, independent of oxidative status.

In general, studies showed that high pH (alkaline) solutions, such as sodium chloride, ammonium hydroxide, and sodium hydroxide, offer potential solutions to replace phosphates in the meat enhancement solutions (Parsons et al., 2011a; Parsons et al., 2011b; Rigdon et al., 2017). Using high pH alkaline solutions for enhancement increases the pH of the meat system resulting in increased WHC and improved tenderness and colour. For example, the study by Parsons et al. (2011a) used a brine containing 1% ammonium hydroxide (AHT) in beef strip loins. It demonstrated that phosphates could be replaced as indicated by improved colour and retail display properties. However, due to the increased pH, the microbial load of the AHT strip loins were higher when compared to the control. Hence, the product’s shelf life should be considered when altering the pH. Studies replaced phosphate using rice starch and fructooligosaccharides as substitutes for dextrose in cooked hams. Results showed that rice starch could reduce phosphate content with acceptable product quality. However, a negative effect on appearance, eating quality and cooking yield was observed (Resconi, Keenan, Barahona, et al., 2016a; Resconi et al., 2015).

Regarding oxidative stability, studies by Bao, Ushio, & Ohshima (2008) demonstrated an increase in pH and a decrease in oxidation when 5ml of mushroom extract containing ergothioneine was added to beef and fish meats, thus improving the retail display characteristics. Also, the study of Choe et al. (2018) showed no significant difference between the oxidation of sausages treated with phosphates or winter mushroom powders. Thus, ingredients that do not modify the colour and have some antioxidative activity could contribute toward phosphates reduction in processed meat products.

1.6.4 Dietary Fibres - A special interest as phosphate replacers

Among the various ingredients discussed in Table 1-2, dietary fibres, obtained from multiple fruit and vegetable sources, have gained interest among the meat industries since they can make up the deficit of natural fibre content in naturally high nutritious meat products (Das et al., 2020). Dietary fibres improve meat quality in technological, nutritional and health aspects (Santos et al., 2020). Dietary fibres are proven to reduce the incidence of colon cancers in meat consumers because of their ability to reduce the reaction time between the colon membrane and carcinogenic substances present in the meat. This was explained by the belief that dietary fibres cut short the transit time of faecal matter in the intestine because of their higher water absorption capacity (WAC) (Ahmed, Khalid, & Younis, 2020). Technologically, dietary fibres improve the WHC, oil holding capacity (OHC), cook yield, emulsion stability, juiciness, texture, and prevent lipid oxidation in meat products (Elleuch et al., 2011; Younis et al., 2022). These dietary fibres are categorised as functional foods. They are available in both pure (alginate, carrageenan, cellulose, gum, hemicellulose, inulin, lignin, pectin, and so on) and composite (bran, peels, pomaces and by-products) forms. These intrinsic properties of dietary fibres have encouraged the researchers to use fibre-rich ingredients such as sugar beet (Ağar et al., 2016), pineapple fibre (Henning, Tshalibe & Hoffman, 2016), corn bran, apple and tomato pomace (Yadav et al., 2016), sugarcane fibre (Fang et al., 2019), dragon fruit peel (Madane et al., 2020) and Mosambi peel (Younis, Ahmed, & Malik, 2021) in processed meat products, thus justifying the choice to utilise them as phosphate replacers. Apple pomace (AP) and coffee silverskin (CSS) are the two dietary fibre-rich ingredients of interest as phosphate replacers in meat, discussed in this thesis review.

1.6.4.1 Apple Pomace

Apple pomace (AP) is the main solid residual by-product of apple juice processing industries (Figure 1-3). AP is rich in moisture, total dietary fibre (TDF) and bioactive components like polyphenols, antioxidants, minerals and vitamins (Younis & Ahmed, 2018). Various polyphenols in apple pomace are responsible for their excellent antioxidant properties. Apple pomace comprises a higher TDF of 62.67% in the dry powder form, with 54.12% as insoluble fibre and 8.55% as soluble (Younis & Ahmed,

2015). Incorporating this TDF-rich apple pomace in meat products could help in increasing the WHC, OHC, and emulsion stability properties. Numerous studies have reported the functional usage of apple pomace in meat and processed meat products. Studies by Rather et al. (2015) on the fat-replacing ability of apple pomace in goshtaba (a traditional meat-based product in Jammu & Kashmir, India) showed that the application of apple pomace improved the cooking yield and the antioxidant properties while decreasing the textural hardness. In another study by Younis & Ahmad (2015), apple pomace powder obtained from apple processing, used as a functional ingredient in buffalo sausages, effectively improved the emulsion stability, water activity, and cooking yield. Similarly, the studies of Younis and Ahmed (2018) on the application of apple pomace on buffalo meat patties significantly improved the WHC, cooking yield, emulsion stability, thickness, and textural parameters. This increase in their physical properties was attributed to their higher dietary fibre content, thus, proving that apple pomace can replace phosphates in processed meat products.



Figure 1-3: Dried (left) and powdered (right) form of apple pomace sourced from Muns Agroindustrial S. L. (Lleida, Spain).

1.6.4.2 Coffee silverskin

Coffee silverskin (CSS) is the thin tegument outer layer surrounding the coffee bean seeds obtained as a by-product of the coffee roasting process. Like AP, CSS, in dry powder form (Figure 1-4), is high in total dietary content of about 60-86%, comprising about 50% insoluble and 15% soluble fibres (Iriondo-DeHond et al., 2019). The mineral composition analysis has shown that CSS consisted of approximately 1.5g/kg of natural phosphorus. CSS also possesses biological antioxidant activity. It was

reported that CSS could prevent the development of cancer cells due to a high quantity of polyphenols, mainly melanoidins, which improve their scavenging properties (Saratale et al., 2020). CSS has a lower content of lipids and carbohydrates and consists of a good amount of relevant amino acids, making it easy to incorporate into food preparations (Machado et al., 2020; Martuscelli et al., 2021). Numerous studies have reported the application of CSS in various food products such as bread (Pourfarzad et al., 2013), biscuits (Garcia-serna et al., 2014), nutraceutical yoghurt (Bertolino et al., 2019), gluten-free bread (Guglielmetti et al., 2019), to name a few. Very few or no studies have reported their application in meat and processed meat products. However, the higher TDF content of CSS could make them a suitable replacer for phosphates in meat products.



Figure 1-4: Dried (left) and powdered (right) form of coffee silverskin sourced from Illy caffé S. p. A (Trieste, Italy).

1.6.4.3 Benefits of utilisation AP and CSS

Applying fibre-rich food by-products ingredients AP and CSS in meat helps the meat industries address the clean label requirements. The high fibre content of the ingredients could make up for the natural fibre deficit in meat products and decrease the risks of colon cancer. Besides the nutritional benefits, valorisation of these fibre-rich agro-based food by-products helps the food industries manage the food waste issues, thereby helping develop a better sustainable environment (Younis and Ahmed, 2018; Martuscelli et al., 2021).

1.7 Processing technologies for phosphate reduction in processed meat

The consumer demand for high quality and less processed foods with minimal ingredients and additives has resulted in the shift towards innovative non-thermal clean processing technologies like power ultrasound (US), high pressure (HPP), plasma technology, pulsed X-ray, ultrafiltration and electrical methods. These non-thermal technologies can overcome the disadvantages of thermal technologies by maintaining the sensory and nutrient value and ensuring microbial safety of processed foods (Inguglia et al., 2017). The mechanisms of some technologies could assist in phosphate reduction in meat products when used alone or in combination with phosphate alternative ingredients. Cold atmospheric plasma, pulsed UV light and ozone are generally used for surface treatment, mainly for surface decontamination of pathogens in meat products (Troy et al., 2016). Pulsed electric fields (PEF) and Shockwave (SW) are two emerging technologies for meat application. Both technologies can potentially rupture the meat matrix and thereby improve ingredient interaction with the proteins. Toepfl, Heinz and Knorr (2006) demonstrated that PEF could improve the WHC, yield and texture of injected hams containing phosphate. While the study's objective was not to reduce the phosphate, phosphate reduction may have been possible with PEF without affecting quality. Similarly, while SW has not been assessed directly for phosphate removal, in a study on sausages containing various levels of salt (1.8-1.9 % or 2.2-2.4 % NaCl), SW treatment reduced the cooking loss by 2% in the 1.8-1.9% NaCl sausages (Heinz, 2014). Recent comprehensive reviews of the mechanisms and potential of PEF and SW for the tenderisation of meat exist (Troy et al., 2016; Warner et al., 2017). However, their application to processed meat is limited. Hence, power ultrasound (US) and high-pressure processing (HPP) will be discussed. The focus is on their interaction with alternative ingredients in creating minimally processed meats with reduced or removed phosphate, a novel approach to cleaner labelled processed meats.

1.7.1 Power Ultrasound (US)

The US is a non-thermal processing technology that uses sound energy of frequencies higher than the human audible range (>20 kHz) and lower than microwave

frequencies (10 MHz). Detailed information on ultrasonic mechanisms and the various physical and chemical mechanisms can be found in several comprehensive reviews (Alarcon-Rojo et al., 2015; Alarcon-Rojo et al., 2019). Studies have been conducted using the US for microbial inactivation in meat (Kang et al., 2017a), meat tenderness (Chang et al., 2015; Warner et al., 2017,) and accelerated meat processing like brining and curing (McDonnell, Lyng, Arimi, & Allen, 2014; Ojha et al., 2016). In terms of the possibility of the US in a phosphate reduction strategy, this could include improved functionality of ingredients for meat application by pre-treatment with the US, improved ingredient distribution within the meat matrix or the effect of the US on meat quality parameters when applied to the manufactured product.

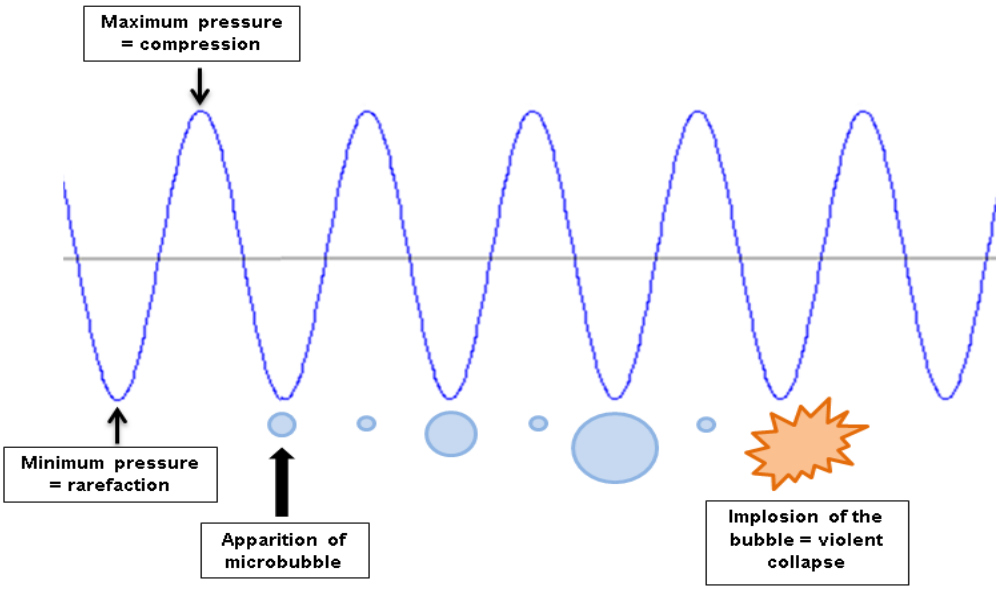


Figure 1-5: Schematic representation of cavitation produced by direct field pressure from US probe.

1.7.1.1 Water binding and ingredient distribution properties

US can modify the WHC and OHC of added alternative ingredients, which could be applied in processed meat manufacture, without any adverse effect on their properties. Studies by Resendiz-Vazquez et al. (2017) showed that there is a significant change in the WHC and OHC of jackfruit seed protein isolates when treated with the high-intensity US for 15 min at 20 kHz with the power input level of 200, 400 or 600 W. Further, Kohn et al. (2016) studied the effects of US on the water

absorption capacity (WAC) of added ingredients. When two groups of ingredients (proteins and polysaccharides) were treated in an ultrasonic water bath at 40 kHz frequency for 15 and 30 min, significant increases in the WAC for polysaccharides were observed. In a recent study, Pinton et al. (2019) found that 18 min of US (25 kHz, 230W) could account for a 50% reduction in phosphate levels in meat emulsions.

Further investigations are required to understand this effect. Still, it could be attributed somewhat to accelerated diffusion as the US has been shown to accelerate mass transfer into the meat matrix. Studies by Ozuna, Puig, Garcia-Perez, Mulet, & Carcel (2013) assessed the application of US on pork brining kinetics and found that the US increased the NaCl and the effective moisture diffusivity. Similarly, McDonnell et al. (2014) proved that meat curing time could be reduced by 50% by operating the US at a pilot scale. There was no significant effect on sonicated meat's quality and sensory properties in the same study. Ojha et al. (2016) also showed that US treatment during pork brining could accelerate the diffusion of a commercially available salt replacer that targets sodium replacement. Thus, the US can accelerate salt diffusion and, possibly, other additives in meat during brining.

Therefore, this combined ability of the US to reduce additive requirements, improve ingredients distribution in meat products and increase the functionality of ingredients could be applied as a hurdle approach toward phosphate reduction in meat products and warrants further investigation.

1.7.1.2 Texture and sensory properties

Applying the US through a biological structure produces compressions and depressions in the microstructure resulting in cavitation (Figure 1-5). Studies have indicated that this results in microstructural changes to the meat matrix (Siro et al., 2009). Several experiments have studied the effect of US on the textural properties of meat (Alarcon-Rojo et al., 2015). As discussed in a review by Warner et al. (2017), the effect of US on meat texture is dependent on many processing parameters. Thus, the results in the literature are variable. When sonicated meat was cooked at 50^{o}C, the texture was soft compared to non-sonicated meat, such that the shear force was reduced by 6N when compared to the control sample. Thus, when meat products are

treated in the US, it could change the textural properties of the meat and increase WHC. The negative impact of the US on meat product characteristics is scarce (Alarcon-Rojo et al., 2019). Similarly, Pinton et al. (2019) found that US efficiency in meat processing depended on processing parameters when applying US (25 kHz, 230W) for 9 or 18 min to meat emulsions. It was found that 18 min of US could offset defects caused by up to 50% phosphate reduction, including increased cohesiveness and higher texture scores in sensory analysis. On the other hand, other authors have found no change to textural properties of meat sonicated during brining. However, they found accelerated diffusion of NaCl (McDonnell et al., 2014). Therefore, there is evidence that the US can reduce additive requirements, improve ingredient distribution, and offset quality defects caused by phosphate reduction. However, the optimisation of several process parameters is required when applying the US to meat.

1.7.1.3 Oxidative stability

US treatment can lead to the formation of free radicals that might accelerate lipid oxidation in meat products. Studies have shown that using the high-intensity US on meat products increases the lipid and protein oxidation that could affect the textural properties (Chang et al., 2015; Kang et al., 2017b; Alarcon-Rojo et al., 2019). However, they can be controlled using various factors like pressure, temperature and US settings (Pinton et al., 2019). In the study by Pinton et al. (2019), there was no increased lipid oxidation when cooked meat emulsions were treated with ultrasonic power of 25 kHz for 9 and 18 mins. Thus, optimisation of processing parameters is essential to maintain quality parameters.

1.7.2 High-Pressure Processing (HPP)

HPP is another important non-thermal processing technology. This involves subjecting HPP food products to very high hydrostatic pressure from 300-600 MPa and mild temperatures (<45^{o}C), which can inactivate micro-organisms and enzymes in food products without any effect on product colour, flavour and nutritional composition (O'Flynn et al., 2014). More detailed information on HPP results on meat products is found in several studies (Hygreeva & Pandey, 2016; Chen et al., 2018).

1.7.2.1 Water binding properties

HPP can cause conformational changes in proteins leading to protein denaturation, aggregation, or gelation, which helps improve the functionality of comminuted meat products. In doing so, HPP also plays a significant role in enhancing the WHC of meat products. Various studies have reported on the effect of HPP on the water-binding capacity (WBC) of meat products (Zheng, Han, Yang, Xu, & Zhou, 2018). The pressurisation of meat products improved the gel-forming properties of meat proteins, thus enhancing the WHC and textural characteristics of meat products. Various studies showed that HPP increased the emulsion stability, chewiness, cohesiveness, hardness, and gumminess and decreased cooking and purge loss in meat products (Inguglia et al., 2017). Studies by Crehan, Troy, & Buckley (2000) assessed the effect of HPP on frankfurters with various salt levels and reported notable improvements in the juiciness and textural properties. Studies have also shown that HPP plays a significant role in replacing additives in meat products by promoting the cohesive properties of meat particles. Heat set gels formed after HPP treatment of comminuted meat products have been shown to have improved characteristics with both low and high salt concentrations (Ikeuchi, Tanji, Kim, & Suzuki, 1992). Grossi et al. (2012) studied the effect of HPP treatment on salt-reduced sausages with carrot fibre and/or potato starch as salt replacers. Pork sausages with different formulations of salt, carrot fibre and/or potato starch were treated with 400, 600, or 800 MPa for 5 minutes at 5 or 40 ^{o}C. Results of WBC tests proved that the incorporation of HPP and a new functional ingredient improved the WHC of low salt sausages to the same level as high salt sausages. The experiment concluded that HPP at 600 MPa could reduce the salt content of hydrocolloid-containing pork sausages from 1.8 to 1.2% without negatively impacting the WBC, texture, and colour. Similar results were obtained when salt-reduced hams were treated with 100 MPa (Tamm et al., 2016).

1.7.2.2 Texture and sensory properties

O'Flynn et al. (2014) investigated the use of HPP on phosphate-reduced breakfast sausages and its effect on physicochemical and sensory characteristics. Sausages with 0, 0.25, and 0.5% phosphate content were manufactured using the raw minced

pork meat, pre-treated with HPP at 150 or 300 MPa for 5 minutes. It was found that HPP-treated phosphate-free sausages had improved emulsion stability compared to the non-HPP-treated control. However, a slight decrease in the juiciness was observed for the sausages treated with HPP. The complete results concluded that the administration of HPP treatment at 150 MPa for 5 minutes positively reduced the phosphate content in low-fat breakfast sausages to 0.25%, without any negative impact on the functional characteristics. Despite various successful results, it was also found that there were some adverse effects on the sensory and acceptability attributes of the meat products. Decreased functional properties in sausages were observed when treated with HPP at 300 MPa (O'Flynn et al., 2014). Application of HPP over 400 MPa reduced the WHC in meat batters, thus affecting the sensory characteristics of the meat product.

1.7.2.3 Colour and oxidative stability

Fuentes et al. (2010) showed that applying high hydrostatic pressure of 600 MPa for 6 minutes increased the lipid and protein oxidation in vacuum packaged Iberian dry-cured ham. Similarly, protein and lipid oxidation increased when HPP of 600 MPa was applied to the cooked and raw ground beef for 5 minutes (Jung et al., 2013). Other disadvantages of HPP are reduced sensory properties due to the resistance offered by food enzymes and pressure-resistant bacterial spores resulting in food spoilage (Inguglia et al., 2017). This highlights the importance of optimising processing parameters.

Nonetheless, the ability of HPP to solubilise and extract myofibrillar proteins and improve WHC and ingredient interaction in meat helps in the reduction of additives like phosphates, thereby claiming the meat products as cleaner labelled healthier meat products. Indeed, there is a lack of studies assessing the interaction of HPP and alternative ingredients as phosphate replacers in meat products.

1.7.3 Cost and Industrial adoption

Understanding the development and application of these processing technologies is very important in terms of their cost and industrial adoption. Apart from consumer acceptance, the industrial adoption of these technologies is largely dependent on

increasing growth, revenue, and productivity (Priyadarshini, Rajauria, O'Donnell and Tiwari, 2018).

1.7.3.1 Power Ultrasound (US)

US processing is a growing superior, novel processing technology with the ability of more considerable commercial scale-up and better payback on capital investment. Recently, US technology has been widely employed in Europe and USA for its application in food processing. Preservation techniques include homogenisation, crystallisation, dispersion, emulsification, plant sanitation, viscosity alteration, solubilisation, filtration, separation, extrusion, fermentations, texture improvement and ultrasound-assisted extraction (UAE) of various biochemicals from plants and foods (Priyadarshini et al., 2018). This advancement in industrial adoption is due to the significant improvement in the efficiency (up to 85%) of generators and transducers that has reduced the system failure. With this improved efficiency and advanced technologies, it has been reported that the US can be easily installed in the existing industrial facility (Patist & Bates, 2011). The application of high-intensity US in the food industry is highly dependent on the availability of low-cost instrumentation (Rastogi, 2011). It has been reported that the US, among the various novel processing technology, has low installation and maintenance costs (Patist & Bates, 2011). This is highly due to the robustness of instruments without any moving and complicated parts. The US probe (sonotrode) of lifetime 3-24 months is the only replaceable part employed in the technology. This allows the easier adoption of the US by the food industries. However, the application of the US is still limited to laboratory scale in terms of meat processing, and the future commercial application will hugely depend on addressing the challenges like developing standardised methodology and control parameters. There should be vigorous testing before application to prove the technology's safety (Rastogi, 2011). The absence of sensory analysis of meat products with US-treated ingredients in the later chapters of this thesis could be explained by this uncertainty over the safety of US-treated food ingredients and the lack of official regulations.

1.7.3.2 High-Pressure Processing (HPP)

The industrial applications of HPP on foods have been growing exponentially since 1990, producing over 250 different HPP-treated products (Sampedro et al., 2014). It has been reported that the global market value for HPP food products was around $10 billion in 2015, and it is expected to grow up to a market value of $55 billion by 2025 (Huang et al., 2017). Approximately 500,000 tonnes of meat products are being HPP-treated every year worldwide, with ready-to-eat meat products (e.g. burger patties) representing the most. In other words, about 25-30% of the total HPP-treated food products are meat products such as vacuum-packaged ham, bacon and patties (Bolumar et al., 2020). With the increasing global demand, purchasing and installing HPP instruments is a significant factor, which depends on the total cycle time, vessel filling ratio, energy labour and capital costs (Samperdo et al., 2014). It has been estimated that a complete set of HPP would cost approximately $0.5 – 4 million (Balasubramaniam, Barbosa-Cànovas, Lelieveld, 2016). Despite the higher capital investments, the application of HPP is on the rise owing to its various applications and higher efficiency.

1.8 Thesis objectives

Objective 1: To study the consumer purchase intention of phosphate reduction in processed meat

- To examine the consumer awareness of the purpose of phosphates in processed meat products using the Theory of Planned Behaviour (TPB)
- To measure the importance of phosphate reduction in processed meat products from a consumer perspective

Objective 2: to optimise the phosphate-reduced sausage formulations

- To determine the phosphate replacing ability of the phosphate alternatives
- To determine the minimum phosphate requirements and optimum combinations of phosphate alternatives

Objective 3: To improve the techno functionality of the phosphate alternative ingredients

- To improve the functionality of the phosphate alternatives using US technology
- To optimise the US treatment parameters for the improved performance

Objective 4: to improve the quality of phosphate-reduced sausages using novel processing technologies

- To apply HPP treatment to the meat before product manufacture
- To utilise the US-treated phosphate alternatives in the sausage formulations
- To determine the best formulation with reduced phosphate and higher quality.

Note - The sensory analysis of the meat products was not carried out owing to the safety concerns over the consumption of US-treated food ingredients.

Chapter 2. Consumer intention study towards the phosphate reduced processed meat products using the extended theory of planned behaviour

Overview

This chapter investigates the consumer perception of purchasing phosphate-reduced processed meat products. A survey study was employed to measure the purchase intention using the extended Theory of Planned Behaviour (TPB). Participants (n=548) from across the Republic of Ireland (ROI) participated in this study, and their response to the questionnaire was analysed to measure their knowledge and attitude towards phosphate reduction. Results from this chapter have been submitted to a peer-reviewed journal for publication.

- **Thangavelu, K. P.**, Hyland, J. J., Henchion, M., Kerry, J. P., & Álvarez, C. Consumer intention towards the phosphate-reduced processed meat products using the extended theory of planned behaviour. *Meat Science, 193,* 108947.

2.1 Introduction

For centuries, meat and meat products have been an integral part of the global human diet (Kiran et al., 2018), and it is expected to continue to be necessary, with indications that consumption of meat will grow in developing countries in the coming decades (Llauger et al., 2021). According to Henchion et al. (2021), the average global demand for animal-based food products in 2050 will increase by approximately 2.0 billion tonnes, with the current level of per capita consumption. This is partly due to the high nutritious value of meat which is a good source of protein, essential amino acids, vitamins, and minerals such as vitamin B, vitamin A, zinc and iron (Rohrmann & Linseisen, 2016). However, various adverse health impacts and environmental issues could be perceived as this increased meat consumption, mainly processed meat products. This processed meat includes value-added products prepared using portioning, forming and processing techniques such as emulsification, salting, curing, marinating, cooking, smoking or drying techniques (O'Farrell, 2011). These negative consumer perceptions of processed meat products are due to high sodium levels, fat, and harmful additives (Shan et al., 2016). Consumers' perceptions of the health aspects of processed meat products are the primary factor determining their consumption. In recent years, there has been a growing consumer demand for healthy and natural meat products, which was strongly influenced by the news media's significant attention to the reduction of processed meats mainly because of their perceived carcinogenic effects (Henchion & Zimmermann, 2021; Sievert et al., 2022). Consumers are more aware of the negative health impacts of consuming high salt levels, high saturated fat levels, curing salts, etc. These have been linked to processed meat consumption. They are also aware of the industry's alternative ingredient strategies in the whole or partial replacement of these meat processing aids, such as low-salt (KCl), seaweed, plant fibre, plant extracts, etc. The 'clean-label' strategy is one such industrial approach possessing some consumer comprehension (See Chapter 1, Section 1, page 4). With no official definition, clean label products are those with a shortlist of non-synthetic, natural ingredients (Delgado-Pando et al., 2021). These trends have resulted in the reduction/removal of additives in processed meat products.

Phosphates are a processing aid or additive used in processed meat and poultry products. They can enhance physical processing, water binding, protein functionality, texture and flavour in final products. Phosphates in the meat industry are used as sodium and potassium salts of phosphoric acid. They are used as pH adjusters, buffers, sequestrants, acidulates, bases, gel accelerants, dispersants, precipitants and ion-exchange agents in processed meat products (See Chapter 1, Section 1, page 3). Phosphates can fixate the free water into bound water by dissociating myofibrillar meat proteins and increasing the meat's pH and ionic strength (Casco et al., 2013). They can also reduce salt usage in meat products without affecting technological and sensory quality (Moiseev & Cornforth, 1997; Resconi et al., 2016). Due to these effects, meat proteins can maximally exert emulsifying and gelling properties, which are essential concerning water holding capacity (WHC) and fat emulsification (Glorieux et al., 2017).

It has been reported by the European Food Safety Authority (EFSA) that almost 17 different food categories have been authorised at the EU level to use phosphate additives (Authority, 2013). The acceptable daily intake (ADI) of phosphates for a healthy adult is 40 mg/kg, expressed in Phosphorus (P), and the study by Leon, Sullivan, and Sehgal (2013) showed that processed food contributed to an extra 700-800mg of P intake per day. Kidneys in a healthy adult control this consumed excess phosphorus; however, it may increase blood phosphate levels, called hyperphosphatemia in patients with chronic kidney disorders (Vervloet & van Ballegooijen, 2018). Additionally, increased consumer interest in clean food labelling has resulted in a decline in phosphates in meat processing due to the negative perception of their artificial and inorganic status (Petracci et al., 2013). This signifies the necessity to remove phosphates from processed meat products. However, the complete removal of phosphates from processed meat products produced negative quality characteristics (Glorieux et al., 2017). Hence, attempts have been made to reduce the phosphate concentration using natural alternative ingredients (Câmara et al., 2020; OSullivan et al., 2004) and novel food processing technologies such as ultrasound (US) (Pinton et al., 2019) and high-pressure processing (HPP) (Maksimenko et al., 2019). However, using these techniques to reduce phosphates

can produce positive or negative changes in processed meat products' eating and other sensory characteristics (e.g. appearance). Furthermore, given the likelihood that reducing phosphates will influence the product and the process. With the possibility of higher costs, the industrial adaptation of these reduction techniques will be affected by consumer acknowledgement (Teixeira & Rodrigues, 2021). There is significant scientific evidence of consumer rejection of such products. For example, a study by Paglarini et al. (2019) reported lower consumer acceptability of an emulsion gel based on soy oil to replace pork fat in frankfurters. Hence, studying the consumer acceptance of phosphate reduction in processed meat products is essential. However, several scientific studies reported phosphate reduction in processed meat products, and very few or no studies have been written on consumer perception and purchase intention towards them. Therefore, the objective of this study was to determine the consumer purchase intention towards the phosphate reduced processed meat products using the well-established Theory of Planned Behaviours (TPB) model that measures the attitude, subjective norm, and perceived behavioural control, with a modification that includes a perceived health risk construct associated with its consumption.

2.2 Conceptual model

The conceptual framework of this survey study was based on the modified Theory of Planned Behaviour (TPB). As an introduction, TPB was originally the extended derivative of the Theory of Reasoned Action. The Theory of Reasoned Action was widely used to study the relationships between consumers' beliefs, attitudes, and behaviour (Fishbein & Ajzen, 1975). The influence of attitude on behaviour is predicted by behavioural intention. The intention referred to the consumers' interest in performing the behaviour. It was expected using the variables (i) attitude - personal attitude to the behaviour and (ii) subjective norm - perceived social pressure from others to perform the behaviour (Guàrdia et al., 2006). However, the Theory of Reasoned Action did not predict the behaviour of all classes. This issue of 'incomplete volitional control' posed by the Theory of Reasoned Action was addressed by the TPB developed by Ajzen (1985). The addition of Perceived Behavioural Control (PBC) to the theory of reasoned action produced the TPB. PBC

represents consumers' perception of the extent to which the individual feels it is easy or hard to adopt the behaviour. In 1991, the modified model of TPB was proposed by Ajzen (1991), which removed the belief construct from the model. Ever since, TPB has been one of the most frequently used models to explain and understand human social behaviour towards the discussed topic (Ajzen, 2011). Numerous researchers have used the TPB model to understand consumers' purchasing and healthy eating behaviour intentions (Al-Swidi et al., 2014; Cornélio et al., 2012; Dorce et al., 2021; Grønhøj et al., 2013; Guàrdia et al., 2006; Lim & An, 2021; Wolstenholme et al., 2021). Therefore, this study employed an extended TPB to measure consumers' intentions to purchase phosphate-reduced processed meat products.

2.2.1 Theory framework and hypotheses

TPB comprises constructs such as attitude, subjective norms, and PBC, which influence consumers' purchase intention. Furthermore, we modified the TPB model in this research study by including the potential influencing construct 'Perceived Health Risk' (Figure 2-1).

2.2.1.1 Attitude hypothesis (H_1)

Attitude generally refers to the degree of evaluation towards the behaviour in either a positive or a negative way (Ajzen, 1991). In this study, attitude represents the consumer purchasing behaviour towards phosphate-reduced processed meat products. A study by Ding et al. (2022) showed that a person with a positive attitude positively influences behavioural intention. Thus, consumers are more likely to purchase phosphate-reduced processed meat products with a positive attitude. Therefore,

H_1: Attitude significantly impacts the consumers' purchase intention towards phosphate-reduced processed meat products.

2.2.1.2 Subjective norm hypothesis (H_2)

Subjective norms refer to the individual's perceived social pressure by the opinion of significant others, such as family and friends in their social group, to perform the behaviour (Ajzen, 1991). A study by Chen and Tung (2014) reported that the person's inclination to perform a given behaviour significantly increases with increased social

pressure. In our study, the subjective norm measures the social pressure received from friends and family of the consumers to purchase phosphate-reduced processed meat products. Therefore,

H_2: Subjective norms significantly impact the consumers' purchase intention towards phosphate-reduced processed meat products.

2.2.1.3 PBC hypothesis (H_3)

PBC refers to the perceived ease or difficulty the individual faces in performing a specific behaviour of interest (Ajzen, 1991). Like other constructs, positively biased PBC positively influences behavioural intention, i.e., increased availability of resources and opportunities will significantly affect the purchasing intention of phosphate-replaced processed meat products (Ding et al., 2022). Therefore,

H_3: PBC significantly impacts the consumers' purchase intention towards phosphate-reduced processed meat products.

2.2.1.4 Perceived health risk hypothesis (H_4)

Perceived health risks are the perception of individual risks developed by performing the behaviour (Ritchie et al., 2021). It is not a conventional construct of the TPB but is incorporated into the modified model used in this research. This was due to its importance concerning consumer purchasing behaviour about phosphates products. This study evaluated the perception of the risks involved in the employment of added phosphates in processed meat products. Hence, the higher the health risk perception, the higher will be the chances for consumers to perform the purchasing intention of phosphate-reduced processed meat products. Therefore,

H_4: Perceived health risks significantly impact the consumers' purchase intention towards phosphate-reduced processed meat products.

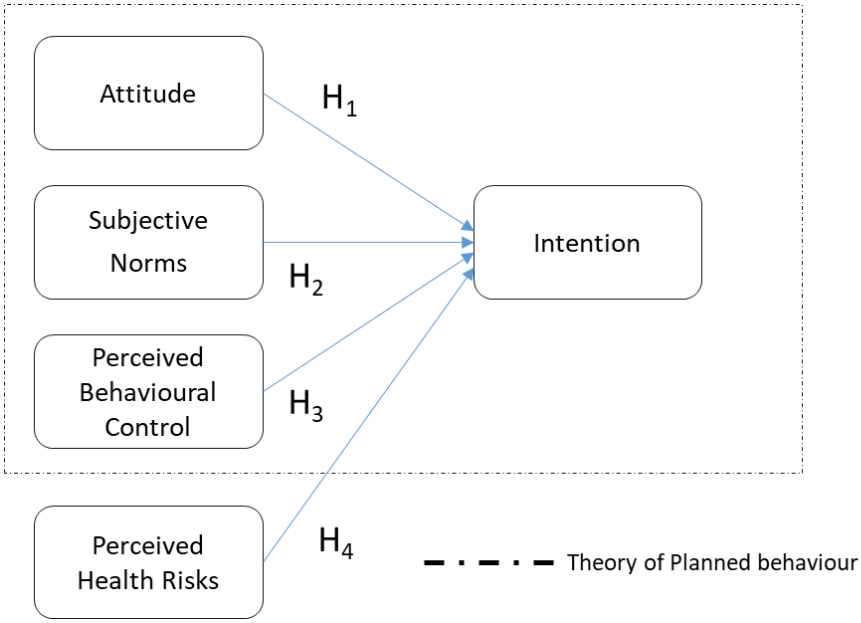


Figure 2-1: Framework of the extended theory of planned behaviour model

2.3 Methods

2.3.1 Data collection

The study adopted a quantitative design, and the data was collected through an online survey conducted among Irish consumers with the help of an external data collection agency. The agency maintains a proprietary research panel of Irish adults living across the Republic of Ireland (ROI) and provides service on the public's opinion across various matters. The data were collected using the My Opinion Matters search engine, and the participants were rewarded with points for survey completion that can be used to claim vouchers. The ethical approval required for the study was obtained from Social Research Ethics Committee (SREC, University College Cork, Ireland). Participants (i) who never or rarely consume meat and meat products, (ii) who work or previously worked in the area of food regulation, food science and technology, or (iii) who stayed in ROI for less than 3-years were restricted from taking the survey. A total of 548-participants completed survey responses from adults (≥18 years of age) from across ROI was obtained from the service provider. Since it was a well-controlled data collection service, unreliable values such as missing or delayed responses were not allowed in the data collected.

2.3.2 Questionnaire development

The survey questionnaire was divided into three sections. The first section comprised sociodemographic questions such as gender, age, region, level of education, current employment, total family income, and social class. The second section involved the questions about the consumers' knowledge and awareness of meat additives and phosphates in processed meat products and their meat consumption characteristics. The third section measured the consumer's behavioural intention towards purchasing phosphate-reduced meat products using the extended variables of TPB. The extended theory in this study consisted of attitude, subjective norms, PBC, and behavioural intention as TPB variables, and perceived health risks as an extended variable.

2.3.3 Measurement of extended TPB variables

2.3.3.1 Attitude

The attitude of consumers towards the consumption of phosphate-reduced processed meat products was measured using four semantic adjectives (e.g. Consuming processed meat products containing a reduced amount of added phosphates is), each on a 7-point unipolar scale (e.g. 1 – highly unsatisfactory to 7 – highly satisfactory) based on the research of Menozzi et al. (2017). The mean value of the items was used to represent the measure of attitude.

2.3.3.2 Subjective norm

The measure of subjective norms influencing the purchase intention of processed meat products containing a reduced amount of added phosphate was assessed using three items (e.g. friends who influence my purchase decisions think I should buy processed meat products with reduced levels of added phosphates), each on a 7-point unipolar Likert scale based on the research of Wolstenholme et al. (2021).

2.3.3.3 Perceived behavioural control (PBC)

The ease of purchasing or consuming the phosphate-reduced processed meat for the consumers was measured using the three items (e.g. Processed meat products with reduced levels of added phosphates are not widely available) of PBC developed based on the research of Elliott et al. (2007), using a 7-point unipolar scale.

2.3.3.4 Perceived health risks

The perceived health risks constructs were measured using the three items (e.g. I believe that the regular consumption of processed meat products with added phosphates is unhealthy) developed based on the research of Bearth et al. (2014), using a 7-point unipolar scale.

2.3.3.5 Intention

Consumers' intention to purchase processed meat products with reduced phosphates was measured using three items (e.g. I expect to purchase processed meat products with reduced levels of added phosphates in the next month) using a 7-point unipolar Likert scale, developed based on the report of Carfora et al. (2017). The measurement items of all variables are present in Table 2-1.

2.3.4 Statistical analysis

Statistical data analyses were conducted using the IBM Statistical Package for Social Science (SPSS, version 27). The consumer's knowledge and awareness of meat additives, especially phosphate additives, in terms of sociodemographic characteristics such as gender, age groups and the highest level of education were analysed using the Chi-square analysis followed by Bonferroni correction (MacDonald & Gardner, 2000; Sharpe, 2015). Regarding the TPB constructs, the internal consistency of the data was verified using Cronbach's alpha. The TPB model hypotheses were validated using multiple regression based on attitudes, subjective norms, PBC and perceived health risks to measure the intention towards consuming phosphate-reduced processed meat products (Francis et al., 2004).

Table 2-1: Measurement of TPB variables

Variable Constructs	7-point scales	Reference
Attitudes		
1. Consuming processed meat products containing a reduced amount of added phosphates is	0 - Highly unsatisfactory to 7 – Highly satisfactory	Menozzi et al. (2017)
2. Consuming processed meat products containing a reduced amount of added phosphates is	0 - Very unimportant to 7 – Very important	
3. Consuming processed meat products containing a reduced amount of added phosphates is	0 – Highly disadvantageous to 7 – Highly advantageous	
4. Consuming processed meat products containing a reduced amount of added phosphates is	0 – Very unacceptable to 7 – Very acceptable	
Subjective Norms		
1. Friends who influence my purchase decisions think I should buy processed meat products with reduced levels of added phosphates	0 – strongly disagree to 7 – strongly agree	Wolstenholme et al. (2021)
2. Family members who influence my purchase decisions think I should buy processed meat products with reduced levels of added phosphates		
3. I feel I am under social pressure to buy processed meat products with a reduced level of added phosphates		
Perceived Behavioural Control		
1. Processed meat products with reduced levels of added phosphates are not widely available	0 – strongly disagree to 7 – strongly agree	Elliott et al. (2007)
2. I do not know how to distinguish processed meat products with reduced levels of added phosphates from conventional ones in the supermarkets, butchers or other outlets		
3. I am confident that I could identify processed meat products that have reduced levels of added phosphates in the supermarket, butchers or other outlets		
Perceived Health Risks		
1. I believe that the regular consumption of processed meat products with added phosphates is unhealthy	0 – strongly disagree to 7 – strongly agree	Bearth et al. (2014)
2. It is not a safe strategy for the meat industry to use added phosphates in processed meat products		
3. I think that processed meat products containing reduced levels of added phosphates are safer to eat		
Intention		
1. I expect to purchase processed meat products with reduced levels of added phosphates in the next month	0 – strongly disagree to 7 – strongly agree	Carfora et al. (2017)
2. I want to purchase processed meat products with reduced levels of added phosphates in the next month		
3. I intend to purchase processed meat products with reduced levels of added phosphates in the next month		

2.4 Results

2.4.1 Sociodemographic characteristics

The results of the sociodemographic information characteristics of the survey participants are presented in Table 2-2. The sample (n=548 panellists/consumers) broadly reflected the adult population in Ireland, obtained from a national census survey by Central Statistics Office (CSO) in 2016, with female (51.0%) and male (49.0%) population distribution. Results showed that most participants were aged between 35 and 44 years (24.8%), reflecting the adult Irish population data (35-44 years - 19.9%). Most of the population were well educated, with an undergraduate degree (31.4%) as their highest level of education and Post-secondary qualification (26.1%) being the next highest.

2.4.2 Meat eating and knowledge characteristics

Only meat-consuming consumers were permitted to be involved in the study. Of this meat-eating cohort, it was found that they generally consumed meat quite frequently, with only 1.6% consuming meat less frequently than weekly. Almost two-thirds consumed meat a minimum of 5-6 times per week (See Table 2-3 for further details). The consumption frequency for processed meat products like; sausages, burgers, hotdogs, pastrami, bacon/rashers, smoked/cured ham slices, pre-cooked roast beef slices and pre-cooked corned beef slices are also provided in Table 2-3. Daily, smoked/cured ham (2.7%) was the most consumed product, followed by bacon/rashers (1.3%) and sausages (0.7%) from the listed processed meat products. An overall comparison of the listed processed meat products showed that sausages had the highest consumption rate (94.9%), followed by bacon/rashers (94.5%), burgers (91.8%) and smoked/cured ham (87.2%).

The Bonferroni corrected Chi-square analysis of the statements used to access the consumer's knowledge of food additives are presented in Table 2-5. The results (Table 2-5: Q1 & Q2) indicated that more than 50% of consumers could determine the major purpose of food additives in processed meat products. Additionally, about 50% of participants rated their knowledge of food additives in processed meat products as moderate or above. Most respondents appeared to understand the functional role of additives in meat products, with 75% of participants agreeing that

food additives are added to increase the shelf-life of the products. In comparison, only 11.9% agreed that they are added to treat, cure or prevent certain deficiencies or diseases such as cancer, which is not the purpose of meat additives. Gender had a significant effect on perceptions of the purpose of phosphates (i) to improve the colour and flavour (male – 66.8%; female – 57.5%) and (ii) to improve the stability and appearance of the product (male – 57.5%; female – 47.2%) whereas age groups significantly affected the purposes (i) to increase the shelf-life (18-24–50.0%; 25-34 – 64.5%; 35-44 – 73.5%; 45-54 – 74.0%; 55-64 – 85.0%; 65 or above - 82.4%) and (ii) to increase the weight of the products (18-24 – 14.3%; 25-34 – 27.4%; 35-44 – 26.5%; 45-54 – 27.5%; 55-64 – 41.0%; 65 or above – 20.9%). No significant difference was observed in the results of the purpose of food additives by the highest level of education. It was also observed (Table 2-5: Q7) that at least 70% of consumers felt that reducing phosphate content in processed meat products was important (5-somewhat to 7-very important). The results showed that the outcome 'important' (point 6 on the Likert scale) was significantly affected by the highest level of education achieved. Participants with primary education (60.0%) had a higher impact ($P<0.05$) compared with the participants with other educational qualifications. Further, the gender and age groups did not affect the outcome of the importance of the phosphate reduction except for the outcome 'very unimportant' (point 1 on the Likert scale) in which gender (male - 0.4%; female – 5.0%) played a significant part. On comparing the results of other knowledge statements, any gender, age group, or educational qualifications did not significantly impact the outcome.

2.4.3 Data reliability and hypothesis tests

The internal consistency of the data obtained for the TPB construct items was verified using Cronbach's alpha coefficient (Cronbach, 1951). The total alpha coefficient of the TPB construct questionnaire was 0.80. The multiple linear regression analysis using the direct measures of TPB constructs showed that the model had a predictive capacity of adjusted R^2=0.47 (adjusted R^2=0.46) for the behavioural intention toward purchasing phosphate-reduced processed meat products. The collinearity statistics and the scatterplot of standardised predicted values versus standardised results proved that the model adhered to the necessary assumptions (multicollinearity,

linearity, homogeneity of variance, independence, and normality) for multiple linear regression. Table 2-4 and Figure 2-2 present the hypothesis test results using multiple linear regression. Results for hypothesis 1 (H_1) that attitude has a significant impact on consumers' purchase intention towards phosphate-reduced processed meat products were supported by t-values of 9.06 and β=0.35. Similarly, hypothesis 2 (H_2) that the subjective norm significantly impacts consumers' purchase intention was again endorsed by the t-values of 7.59 and β=0.26. However, on analysing hypothesis 3 (H_3), no significant relationship between PBC and intention was found (t-values of 1.90 and β=0.06). Hypothesis 4 (H_4) that perceived health risks significantly impact consumers' purchase intention was supported by t-values of 7.52 and β=0.30.

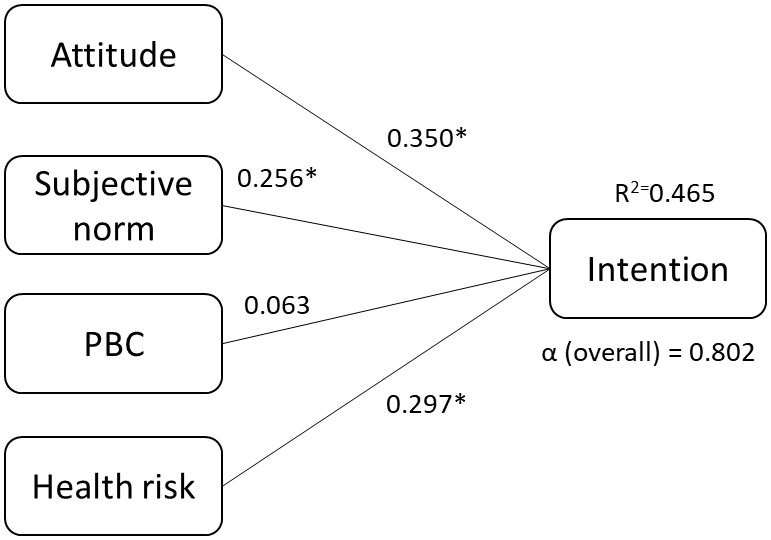


Figure 2-2: Final extended TPB model constructs with standardised coefficients obtained from the results of multiple regression where *P < 0.001.

Table 2-2: Sociodemographic characteristics of the survey participants (n=548)

Variable	Category	Sample (%)	Irish Population (%) (from 2016 National Survey by Central Statistics Office)
Gender	Male	41.2	49.0
	Female	58.8	51.0
Age group (years)	18-24	5.1	15.3 (15-24 years)
	25-34	11.3	17.6
	35-44	24.8	19.9
	45-54	23.9	16.7
	55-64	18.2	13.5
	65+	16.6	17.0
Highest Level of education	Primary	2.7	8.9
	Secondary	25.2	27.3
	Post-secondary (certificate or diploma)	26.1	16.2
	Undergraduate degree (Bachelors)	31.4	15.1
	Postgraduate degree (Masters, PhD)	14.1	8.33
	Prefer not to say	0.5	5.3
Region	Urban City	18.2	
	Urban Town	21.7	
	Suburban	25.4	
	Rural Village	15.1	
	Rural Countryside	19.5	
Current Employment	Full time (≥35 hours per week)	40.3	
	Part-time (< 35 hours per week)	16.4	
	Self-employed	5.1	
	Unemployed and looking for work	2.6	
	Looking after home/family	12.0	
	Student	2.7	
	Retired	17.0	
	Unable to work	3.8	
Total household annual income (before tax & other deductions)	Less than €30,000	25.5	
	€30,000 - €59,999	36.2	
	€60,000 - €89,999	16.9	
	€90,000 - €119,999	6.6	
	€120,000 - €149,999	2.6	
	€150,000 or more	1.3	
	Don't know or prefer not to say	10.9	
Social Class	AB (Upper middle & middle class)	23.2	
	C^1 (Lower middle class)	33.0	
	C^2 (Skilled working class)	12.2	
	DE (Working & non-working class)	30.5	
	F (Farmers & farm labours)	1.1	

Table 2-3: Frequency distribution percentage of consumption rate of processed meat products

Processed meat products	Sample Consumption rate (%)						
	Daily or more often	5-6 times per week	2-4 times per week	Only once per week	1-3 times per month	Less than once per month	Never
Sausages	0.7	1.6	19.9	34.9	21.7	16.1	5.1
Burgers	0.2	0.7	6.0	27.2	33.6	24.1	8.2
Hot dogs	0	1.5	1.6	6.2	11.9	33.9	44.9
Pastrami	0.4	0.2	1.4	4.2	6.4	22.1	65.3
Bacon/rashers	1.3	2.7	21.7	30.5	22.8	15.5	5.5
Smoked/cured ham slices	2.7	6.0	23.9	19.5	16.6	18.4	12.8
Pre-cooked Roast beef slices	0.2	0.9	6.2	10.9	14.1	26.8	40.9
Pre-cooked Corned beef slices	0	1.1	3.8	8.0	13.9	26.8	46.4

Table 2-4: Results of multiple regression of TPB constructs

Hypothesis	Path	Standardised Path Coefficients (β)	t-value	Results
H_1	Attitude -> Intention	0.35	9.05*	Supported
H_2	Subjective norm -> Intention	0.26	7.59*	Supported
H_3	PBC -> Intention	0.06	1.90	Not Supported
H_4	Perceived health risk -> Intention	0.30	7.52*	Supported

Table 2-5: Understanding the participant's knowledge of food additives and phosphates added in processed meat products in terms of gender, age and the highest level of education

Questionnaire n=548	Total (%)	Gender (%)		Age group (in years) (%)						Highest level of education (%)				
		Male	Female	18-24	25-34	35-44	45-54	55-64	65+	Primary	Secondary	Post-secondary	Undergraduate	Postgraduate
1. Purpose of food additives in processed meat products														
Purpose 1	75	71.7	77.3	50.0 [a]	64.5 [a,b]	73.5 [a,b,c]	74.0 [a,b,c]	85.0 [c]	82.4 [b,c]	53.3	67.6	76.8	80.3	78.9
Purpose 2	61.3	66.8 [a]	57.5 [b]	67.9	51.6	54.4	57.3	70.0	72.5	66.7	54.7	69.0	58.4	65.8
Purpose 3	51.5	57.5 [a]	47.2 [b]	42.9	41.9	47.8	50.4	57.0	61.5	40.0	43.2	54.9	56.1	53.9
Purpose 4	27.9	24.8	30.1	14.3 [a,b]	27.4 [a,b]	26.5 [a,b]	27.5 [a,b]	41.0 [b]	20.9 [a]	20.0	28.8	31.0	27.7	22.4
Purpose 5	27.7	31.9	24.8	35.7	24.2	26.5	24.4	34.0	27.5	20.0	24.5	27.5	30.6	28.9
Purpose 6	21.2	21.7	20.8	10.7	14.5	23.5	22.9	24.0	19.8	13.3	15.8	25.4	21.4	23.7
Purpose 7	11.9	15.0	9.6	14.3	12.9	11.8	8.4	12.0	15.4	13.3	12.9	13.4	10.4	10.5
2. Rate your level of knowledge of food additives in processed meat products														
1-Extremely Poor	5.8	5.8	5.9	10.7	6.5	5.9	4.6	3.0	8.8	6.7	8.6	1.4	5.8	9.2
2-Poor	26.5	25.7	27.0	17.9	32.3	27.2	18.3	31.0	30.8	33.3	32.4	25.4	24.3	21.1
3-Satisfactory	17.7	15.9	18.9	7.1	17.7	17.6	18.3	18.0	19.8	20.0	17.3	20.4	16.2	15.8
4-Moderate	31.8	35.0	29.5	42.9	24.2	29.4	38.2	31.0	28.6	33.3	21.6	38.0	34.7	31.6
5-Good	15.9	14.6	16.8	17.9	14.5	17.6	16.8	17.0	11.0	6.7	17.3	12.7	17.3	18.4
6-Excellent	2.2	2.7	1.9	3.6	4.8	1.5	3.8	0.0	1.1	0.0	2.9	2.1	1.7	2.6
7-Outstanding	0.2	0.4	0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3
3. Phosphates are added to improve the juiciness, flavour, and/or eating quality of processed meat products														
Strongly disagree	1.8	1.3	2.2	0.0	1.6	1.5	2.3	4.0	0.0	0.0	1.4	2.1	2.9	0.0
Disagree	4.6	5.8	3.7	7.1	3.2	3.7	4.6	6.0	4.4	13.3	4.3	5.6	3.5	3.9
Somewhat disagree	9.1	10.2	8.4	3.6	4.8	11.8	8.4	9.0	11.0	13.3	10.8	7.0	11.0	5.3
Neutral	43.2	39.8	45.7	42.9	53.2	47.8	41.2	36.0	40.7	33.3	48.9	45.8	37.6	40.8
Somewhat agree	27.2	27.0	27.3	35.7	25.8	22.1	26.7	28.0	33.0	20.0	24.5	24.6	30.1	32.9

Agree	11.9	13.3	10.9	10.7	8.1	11.0	13.0	16.0	9.9	20.0	10.1	13.4	11.6	11.8
Strongly agree	2.2	2.7	1.9	0.0	3.2	2.2	3.8	1.0	1.1	0.0	0.0	1.4	3.5	5.3
4. Excess consumption of processed meats containing added phosphates leads to serious health issues in people														
Strongly disagree	1.3	0.9	1.6	0.0	1.6	1.5	0.8	1.0	2.2	6.7	0.0	1.4	1.2	2.6
Disagree	1.3	2.2	1.2	7.1	0.0	0.7	1.5	2.0	2.2	0.0	2.9	1.4	1.2	1.3
Somewhat disagree	4.7	5.8	4.0	10.7	8.1	2.2	3.1	4.0	7.7	6.7	6.5	3.5	4.6	3.9
Neutral	20.6	22.1	19.6	21.4	25.8	25.7	18.3	14.0	19.8	6.7	28.1	21.8	16.2	15.8
Somewhat agree	35.4	38.1	33.5	21.4	35.5	38.2	34.4	36.0	36.3	26.7	25.2	38.0	43.9	32.9
Agree	23.2	22.1	23.9	21.4	21.0	22.1	25.2	21.0	26.4	40.0	26.6	16.2	23.7	26.3
Strongly agree	13.1	8.8	16.1	17.9	8.1	9.6	16.8	22.0	5.5	13.3	10.8	17.6	9.2	17.1
5. Reduction of phosphates in processed meat products such as burgers and sausages can make them dry and unpalatable														
Strongly disagree	0.9	0.9	0.9	3.6	0.0	0.0	2.3	0.0	1.1	0.0	0.7	2.1	0.6	0.0
Disagree	4.6	4.9	4.3	3.6	0.0	5.9	6.1	3.0	5.5	0.0	2.9	4.9	4.0	9.2
Somewhat disagree	10.0	8.8	10.9	10.7	12.9	7.4	14.5	10.0	5.5	0.0	10.1	7.0	13.3	10.5
Neutral	49.5	45.6	52.2	35.7	54.8	51.5	45.0	48	54.9	40.0	54.0	46.5	49.1	48.7
Somewhat agree	23.5	26.5	21.4	42.9	19.4	25.7	16.0	25.0	26.4	40.0	19.4	28.2	24.3	18.4
Agree	9.1	10.2	8.4	3.6	11.3	6.6	13.0	10.0	6.6	20.0	10.1	9.2	6.9	9.2
Strongly agree	2.4	3.1	1.9	0.0	1.6	2.9	3.1	4.0	0.0	0.0	2.9	2.1	1.7	3.9
6. Reduction of phosphates in processed meat products for health benefits is acceptable even when the eating quality of the product is reduced														
Strongly disagree	1.5	1.3	1.6	0.0	0.0	0.0	1.5	2.0	4.4	6.7	1.4	2.1	1.2	0.0
Disagree	5.5	4.9	5.9	7.1	1.6	5.9	4.6	8.0	5.5	6.7	7.2	4.9	5.2	3.9
Somewhat disagree	8.9	8.8	9.0	10.7	6.5	8.8	7.6	12.0	8.8	0.0	11.5	9.2	7.5	9.2
Neutral	38.9	40.7	37.6	39.3	48.4	30.1	42.0	37.0	42.9	13.3	43.2	42.3	32.9	42.1
Somewhat agree	27.6	28.8	26.7	17.9	27.4	30.9	26.0	31.0	24.2	33.3	18.7	26.1	36.4	25.0
Agree	14.2	12.8	15.2	21.4	14.5	20.6	13.0	7.0	12.1	33.3	15.1	11.3	13.9	15.8
Strongly agree	3.5	2.7	4.0	3.6	1.6	3.7	5.3	3.0	2.2	6.7	2.9	4.2	2.9	3.9
7. Importance of reduction of added phosphates in processed meat products														

Very unimportant	3.1	0.4 [a]	5.0 [b]	0.0	3.2	4.4	3.8	2.0	2.2	0.0	3.6	2.1	4.6	1.3
Unimportant	0.9	1.3	0.6	3.6	3.2	0.0	0.8	0.0	1.1	0.0	0.7	0.7	1.7	0.0
Somewhat unimportant	5.7	8.4	3.7	10.7	1.6	8.1	3.1	4.0	8.8	0.0	5.0	6.3	7.5	2.6
Neutral	21.2	24.3	18.9	21.4	27.4	24.3	22.9	14.0	17.6	6.7	25.2	23.9	15.6	22.4
Somewhat important	31.9	35.4	29.5	35.7	32.3	29.4	27.5	36.0	36.3	26.7	30.9	31.0	34.7	31.6
Important	22.4	19.0	24.8	21.4	22.6	22.1	21.4	24.0	23.1	60.0 [a]	20.1 [b]	19.7 [b]	19.1 [a,b]	32.9 [a,b]
Very important	14.8	11.1	17.4	7.1	9.7	11.8	20.6	20.0	11.0	6.7	14.4	16.2	16.8	9.2

Where (i) Purpose 1 – To improve the shelf life; Purpose 2 – To improve the colour and flavour; Purpose 3 – To improve the stability and appearance; Purpose 4 – To increase the weight; Purpose 5 – To improve eating quality; Purpose 6 – To mask any adverse quality aspects of the raw material used; Purpose 7 – To treat, cure or prevent certain deficiency or diseases such as cancer.

(ii) Percentages with superscripts a, b, and c within the question and sociodemographic characteristics sharing different letters are significantly different from each other ($P<0.05$).

2.5 Discussions

This study investigated factors influencing Irish consumers' behavioural intention to purchase phosphate-reduced processed meat products using the extended TPB model. This study analysed four hypotheses and their implications on behavioural intention. Furthermore, the implications, future studies, and study limitations are discussed.

Although there is no clear definition of clean label products, Asioli et al. (2017) reported that a product can be determined as a clean label in two ways where (i) the front label of the products declared "free-from", "organic", or "no additive" and (ii) the back label of the products contains very few, or a shortlist of non-synthetic ingredients. An increasing trend towards clean label products and consumer interest in healthier and high-quality meat products has removed and replaced synthetic and negatively perceived ingredients such as phosphates from the meat and processed meat products. The consumer response (about 70%) towards the statement on the importance of phosphate reduction, as mentioned in section 2.4.2, emphasised the introduction of phosphate-reduced processed meat products in the food market.

The consumers across ROI (n=548) who participated in this study helped understand their perceptions of added phosphates in processed meat products. The assessment of consumers' knowledge of food additives and their purpose in meat products (Table 4 – Q1 & Q2) produced results similar to the knowledge assessment results (objective assessment identical to Q1) obtained by Nadia Shaheen Koyratty et al. (2014), who studied the food additive control intention among selected consumers from Mauritius (n=180). Further, the results of consumers' knowledge of the purpose of added phosphates in processed meat products (Table 4 – Q3-Q7) showed that the consumers' awareness of phosphate additives in Ireland is deficient compared with their awareness of overall food additives, especially salt (Fellendorf et al., 2018). This showed that public campaigns such as Safe Food Advocacy Europe's and other public media campaigns on 'Phosphate Additives' did not effectively reach and inform Irish consumers. This implies that there is a need for measures to increase the consumers' knowledge of phosphate additives since they are essential for creating a demand for 'additive-less meat products' in the marketplace. This was in line with the findings

made by Mørk et al. (2019), which showed that consumers' knowledge and awareness of the negative impacts of salt increased their purchase intention toward the salt-reduced foods among Danish citizens.

Considering the original TPB model, purchase intention is the significant indicator of consumer purchasing behaviour, i.e., if the consumer has a solid intention to purchase the phosphate-reduced meat products, then their intention will be actioned. However, Menozzi et al. (2015) showed that the original TPB model would not be adequate to demonstrate the consumers' intentions towards the various food choices. Hence, the extended TPB model with risk perceptions is the most relevant model for analysing consumers' purchase intentions. Evidence has shown that including risk perception into the original TPB has helped better understand the consumers' food purchase intention (Chen, 2017; Prati et al., 2012; Qin & Brown, 2008). Chen (2017) showed that perceived health risks supported consumer intentions to avoid consuming food containing additives. Our findings show that phosphates' increased perceived health risks positively influenced the purchase intention toward phosphate-reduced processed meat products. Thus, the higher the health risks the consumers perceive on consuming added phosphates, the greater the chances of avoiding it, resulting in a higher purchase intention towards phosphate-reduced processed meat products. Although consumers' knowledge of the health risks associated with phosphates is limited, the perceived health risk variable positively influenced the consumers' purchase intention of phosphate-reduced processed meat products. This confirmed the H_4 employed in this study.

Previous studies (Aitken et al., 2020; Basha & Lal, 2019; Dorce et al., 2021) have shown that attitude is the strongest influencer of consumers' food purchase intentions. Fellendorf et al. (2018) showed that Irish consumers had a positive attitude towards purchasing salt- and fat-reduced meat products. Similar results were observed in this empirical study, where we observed that attitude was a strong indicator significantly influencing the consumers' purchase intention. This implies that consumers have a positive attitude towards phosphate-reduced processed meat products and, thus, successfully confirm the H_1. The importance of other people influencing purchase intention was predicted using subjective norms, and reports by

Guàrdia et al. (2006) have shown that consumer intention to reduce sodium in meat products in Catalonia (Spain) was significantly affected by subjective behaviour. This research also confirmed that subjective norms are a significant predictor and were the strongest influencer of purchasing intention of phosphate-reduced processed meat products among Irish consumers. This was also established in other studies (Asif et al., 2018; Carfora et al., 2019; Ding et al., 2022), where subjective norms were the strongest predictor in determining the intention toward healthy eating behaviour. This implied that the social pressure perceived by Irish consumers could positively affect their purchasing behaviour, thereby confirming H_2.

On analysing the PBC variable, H_3 postulated in this study was not supported, as it was not significant, thus, signifying that PBC does not mediate attention. A similar result was observed by Mørk et al. (2019), where PBC did not significantly affect the purchase intention of salt-reduced food products among the Danish citizens. The study also reported the negative effect of PBC on intention, thereby concluding that consumers who believed they would have no problem reducing their salt intake were less inclined to purchase salt-reduced foods. Interestingly, this study observed a weak but positive correlation between PBC and purchase intention. This implied that although individuals do not believe they have the necessary capabilities to purchase phosphate-reduced processed meat products, they would still intend to purchase them if widely available.

Overall, the extended TPB variables of this study in terms of; attitude, subjective norms, PBC and perceived health risks accounted for 46% of the total variance, which is a reasonably good fit according to review reports (Armitage & Conner, 2001; Sutton, 1998). In a study by Guàrdia et al. (2006), authors showed that consumer intention to purchase salt-reduced meat products in Catalonia (Spain) predicted a total variance of 60% using the TPB study. This was not in correlation with the current research of intention towards phosphate-reduction in processed meat products because of the insignificance of PBC. This variation in PBC results may be due to a higher familiarity status of salt knowledge among consumers than that associated with phosphates, i.e. consumers have discretionary use of table salt. For example, they can add salt or not at the table/cooking. They do not have much choice about

phosphates, thus reducing the effectiveness of the TPB model. However, the adoption of an extended TPB model with a variable perceived health risk had greatly improved total variance and explanatory power of the study. It helped a lot in the better determination of an individual's purchase intention compared to the original TPB model. This concurs with those reported by Chen (2017), where the extended TPB model with perceived risks as an extended construct improved the total variance of the study to avoid the consumption of food additives. Hence, interventions to improve the PBC variable are required to improve the overall results of this study. Thus, the extended TPB model employed in this study suggests that if the variables; attitude, subjective norms, PBC, and perceived health risks were improved, the result might be the improved support for consumers' purchase intention towards phosphate-reduced processed meat products.

2.5.1 Practical Implications and Interventions

Based on the results obtained, various recommendations based on the significant variables should be developed to increase the consumers' level of purchase intention. These recommendations focus on modifying the extended TPB variables, which improves the behavioural intent to purchase phosphate-reduced processed meat products.

The current study shows that most consumers positively intended to purchase phosphate-reduced processed meat products. Policymakers and experts are responsible for converting this positive consumer intention into action (actual purchase behaviour). It should be noted that a few processed meat products, such as sausages and burgers with reduced/removed phosphate content, are available in the Irish marketplace now. However, these product types are not widely available and are not easily recognised by consumers due to a lack of awareness. This lack of production and low level of knowledge and awareness results in the low market pull at present. Several studies have reported the scientific availability of techniques and technologies (Alternative formulation, US, HPP) to reduce added-phosphate concentration in meat products (Pinton et al., 2021). Food safety measures using sustainable, natural, alternate ingredients for commercial utilisation in meat products are required. The industries could adopt the simple ingredient

reformulation approach using the alternate ingredients to reduce the phosphate concentration in processed meats. Scientific studies have reported reforming processed meat products by employing alternate ingredients in processed meat products. For instance, Cruz-Romero et al. (2022) showed that reformulation using alternate ingredients potassium chloride and tapioca starch could improve sodium- and phosphate-reduced low-fat breakfast flavours and texture properties of sausages. Studies have also been reported on consumer acceptance of HPP processed meat products. For example. Sorenson et al. (2011) showed that Irish consumers widely accepted an HPP treatment of 200 MPa on beef-based chilled ready-meals. Bolumar et al. (2021) review showed that the meat industry had widely adopted HPP, and approximately 400,000 tonnes of meat products are HPP processed every year. Similarly, the US has been commonly employed in Europe and USA to process food products. However, no evidence of its application in meat products has been found, and this may be due to current regulatory restrictions around its usage and possibly owing to concerns over the novelty and unavailability of a standardised methodology for use with meat products (Inguglia et al., 2017; Rastogi, 2011). This shows that the above-discussed technologies have scale-up facilities, and they could be employed in the meat industry specifically to reduce phosphate content in processed meat products. This indicates that there is a need for researchers or scientists to engage with the industries to share this knowledge with them and encourage them to develop such products and put them on the market. There is also need for the industries/public health agencies to demonstrate the benefits of such products to the consumers.

This study showed that consumer attitude strongly influenced the purchase intentions of phosphate-reduced processed meat products. Effective production and market strategies to develop a positive attitude could improve behavioural intention. To address the low levels of consumer phosphate knowledge, industries and public health bodies should do more to promote the health benefits of consuming phosphate-reduced processed meat products. More focused public health campaigns and benefits communications with the help of educational and social media would assist in information outreach. As has already been achieved for salt

usage in processed meats in Ireland through combined policy making and industrial action, phosphate reduction in processed meats could benefit from the same commitment and interactions.

This study also showed that subjective norms and perceived health risks positively impacted consumers' purchase intentions. Considering the interventions for subjective norms, policymakers could focus on improving individual consumer attention and gentle, non-invasive exertion of social pressure. Efforts to spread the individual's experience on knowledge, health, and environmental benefits associated with the products of interest to their friends and families would be helpful. Regarding the perceived health risks of consuming processed meat products with added phosphates, creating awareness among consumer groups may increase their perceived risks of phosphates. Like the attitude intervention strategies, effective communication of potentially adverse health impacts associated with meat additives, especially phosphates, is required with proven medical data. This could be achieved by communicating the knowledge of food additive regulations developed within the European Union on Phosphates (Regulation EC 1333/2008).

It should be noted that although the PBC did not significantly affect the intention, a weak positive correlation was found in this research. As discussed earlier, this implies the incapability of consumers to identify the phosphate-reduced processed meat products. The industries could make efforts to distinguish the phosphate-reduced processed meat products by declaring 'Phosphate additive– Free' or 'Additive –Free' on the front label of packaged products. The widespread availability of such products would be helpful in the simple identification of such products by consumers.

It is essential to consider the limitations associated with this study. The main limitation of this study is that its results were based on a single web survey study. Therefore, this study should be considered an initiation point rather than a result. Future research is needed to provide more contextual information on the results, which could be attained through qualitative analysis. In addition, a decline in the resultant percentage of consumers' knowledge assessment can be expected if it was assessed with open-ended objective assessment questions (e.g. Write down the purpose of food additives in processed meat products) rather than self-reported

assessment. However, this in-depth assessment was not considered in this study since it may result in a time-intensive evaluation affecting the overall response of the consumers (Boyce & Neale, 2006).

2.6 Conclusions

This study successfully investigated the Irish consumers' purchase intention towards the phosphate-reduced processed meat products using the extended TPB constructs. Results generated could be helpful for the meat industry to understand current and future consumers' requirements around processed meats and address the health risks associated with phosphate additive use by proactively getting ahead of an oncoming set of problems. The results serve as the initiation point for future research in the meat industry, for researchers to work on more phosphate-reduced processed meat product formulations, and serve as an information source for food policymakers wishing to address phosphate usage in foods and its potential control measures.

Chapter 3. Phosphate replacing potential of apple pomace and coffee silverskin in Irish breakfast sausage using a mixture design approach

Overview

This chapter investigates the ability of two fibre-rich food co-products ingredients, apple pomace (AP) and coffee silverskin (CSS), to replace/reduce the synthetic phosphates added to the Irish breakfast sausages. This study used a specialised response surface methodology – mixture design approach to develop 18 sausage formulations of different sodium tripolyphosphate (STPP), AP and CSS concentrations. Results of physicochemical properties such as water holding capacity (WHC), cook loss, colour, textural properties, low-field nuclear magnetic resonance (LF-NMR), compositional analysis and lipid oxidation values were analysed. Based on the observed results, the software predicted three optimised sausage formulations based on the desirability response method. The following paper has been published in this chapter:

- **Thangavelu, K. P.**, Tiwari, B. K., Kerry, J. P., McDonnell, C. K., & Álvarez, C. (2022). Phosphate replacing potential of apple pomace and coffee silver skin in Irish breakfast sausage using a mixture design approach. *Meat Science, 185*, 108722.

3.1 Introduction

Consumers' demand for high-quality and healthy food products has resulted in various innovations and the emergence of several processing technologies within the food industry. In particular, and in recent years, consumers have been demanding more minimally processed and 'healthier' meat products (Shan et al., 2017). This has resulted in the introduction of clean-label trends in meat processing, where the usage of artificial additives in the product formulation is avoided. One such recent industrial trend is the removal of phosphate from processed meat products (See Chapter 1, Section 1, page 5).

Phosphates are natural inorganic elements found in many food products. They are essential for human health, as they are required to grow, maintain, and repair cells and tissues, signalling, energy transfer, and other important functions (Kalantar-Zadeh et al., 2010). Generally, phosphates act as emulsifiers, stabilisers, sequestrants, and thickeners in food products. In restructured meat products, phosphates help stabilise meat pH, decrease cook loss and help in improving WHC, emulsion stability, texture and sensory qualities, etc. (Nguyen Huynh Bach Son, 2011). However, the usage of phosphates in meat products is perceived as unhealthy as they are labelled on meat product packaging as E-number additives (European Union-approved artificial food additives). There is a misconception among consumers that E-number additives have negative health implications. However, there is a clear direction that phosphates are not recommended for people who have kidney issues, such as Chronic Kidney Diseases (CDK), as they can cause hyperphosphatemia (Hruska, Mathew, Lund, Qiu, & Pratt, 2008; Ritz, Hahn, Ketteler, Kuhlmann, & Mann, 2012). Thus, replacing/reducing phosphates in processed meat products should be essential for meat industries to improve processed meat composition. From an Irish perspective, breakfast-type sausages are one of the leading products currently employing phosphates as a functional ingredient; thus, this product was selected as our experimental model food product.

Phosphates work in synergy with salt to improve myofibrillar protein extraction, improving processed meats' WHC, textural properties, and shelf-life stability. Although it is a huge challenge to remove phosphates from processed meat products,

studies have been made to replace phosphates with natural ingredient sources like starch, proteins and/or fibres (Casco, Veluz, & Alvarado, 2013; Choe et al., 2018; Resconi et al., 2016). However, the complete replacement of phosphates with other natural ingredients may negatively impact product characteristics and emulsion stability (Resconi et al., 2016). Therefore, finding a better alternative source that could mimic phosphate characteristics is relevant for current and future meat processing needs.

One such promising natural ingredient that could replace added phosphates is fibre. Consumption of dietary fibres can reduce the incidence of colon cancer, diabetes, obesity, and cardiovascular diseases (DeVries et al., 2001). Dietary fibres enhance WHC, modify texture, stabilise fat, improve emulsification properties and as a nutritional enhancer in processed meat products (Petracci, Bianchi, Mudalal, & Cavani, 2013). Fibres obtained from wheat, oat, rice bran, bamboo, peach, pea, grape, apple, orange, carrot, citrus, potato, soy and lupin-kernel are commercially available and are commonly used in meat products. Studies have shown that fibres from vegetable sources, like peas and carrots, are used in comminuted meat products to increase the WHC in the cold- and warm-processing conditions (Petracci et al., 2013). Several studies have assessed the usage of fibres in marinated products for reduced cook loss and improved juiciness, tenderness and WHC (Choi et al., 2013; Petersson, Godard, Eliasson, & Tornberg, 2014; Zhuang et al., 2016). Many food by-products have been identified as a source of dietary fibre, and they can be processed with emerging technologies to produce new commercial food products (Mullen, Álvarez, Pojić, Hadnadev, & Papageorgiou, 2015). Therefore, revalorising food by-products as a source of dietary fibre and phosphate replacers is a realistic approach that minimises food waste and generates healthier and more natural meat products.

Apple pomace (AP) powder and coffee silverskin (CSS) powder are fibre-rich food by-product substances that can potentially be used as phosphate replacers in sausage batters. AP is a by-product of apple juice production and contains high dietary fibre, polyphenols, vitamins and organic acids. In powdered form, AP from different apple sources is reported to have about 78.2 to 89.8% of total dietary fibre, with IDF/SDF ratio varying from 4.5:1 to 12.9:1 (Figuerola, Hurtado, Estévez, Chiffelle, & Asenjo,

2005). A study by Choi et al. (2013) showed that AP could contribute to improving WHC and emulsion stability of chicken sausage emulsions. Similarly, CSS is the fibre-rich outermost layer of the green coffee beans obtained as a co-product of the roasting process. Like AP, CSS has a high amount of soluble dietary fibre (~86% of total dietary fibre) and low-fat content, which could act as a suitable phosphate replacing/reducing agent and fibre content enhancer in sausage emulsions. Studies of the mineral composition of both the ingredients showed that both AP (1.4 g/kg) and CSS (1.462 g/kg) (Martuscelli, Esposito, Di Mattia, Ricci & Mastrocola, 2021a) contained a significant amount of phosphorus in their structure. Using AP and CSS as phosphate replacers in breakfast sausages has not been reported yet, and it can prove economical and the best solution to food by-products management issues.

The present study investigated the replacement strategy using a mixture design to optimise a meat product formulation, using breakfast-type sausages as a model product. Mixture design is an advanced form of response surface methodology (RSM) in which factors are the ingredient mixtures, and the responses are their functions in the product. It is a proper statistical method for multiple regression analysis using measurable results (Yolmeh, Khomeiri, & Ahmadi, 2017). The optimal condition(s) or formulation(s) are predicted using the linear, quadratic, special cubic or cubic model methods. The proportional factors help identify/analyse the important factors (Keenan, Resconi, Kerry, & Hamill, 2014). Therefore, the study's main objective was to investigate the impact of AP powder and CSS powder as phosphate replacers, using a mixture design software approach, on technological properties such as emulsion stability, cook loss, colour, texture profile, WHC, lipid oxidation or water mobility.

3.2 Methods and Materials

3.2.1 Sausage Preparation

Fresh pork loins (6.0 < pH >5.4) were purchased from a local butcher (Gleeson Butchers, Dublin, Ireland). The samples were kept under refrigeration at 4 ^{o}C at all stages. The back fat and other visible fats were trimmed off the lean meat and minced individually using a meat mincer (Meat Grinder MG510, Kenwood, UK). The seasoning mix (salt (49.36%), white pepper (7.15%), preservative (sodium

metabisulphite, E223, 4.35%), ground mace (6.49%), ground nutmeg (5.85%), chilli powder (3.25%), yeast extract(9.28%), ground sage (5.19%), ground marjoram (5.19%), ground ginger (2.6%), antioxidant (Sodium ascorbate, E301, 1.29%)) was purchased from Redbrook Ingredient service (Dublin, Ireland). Sodium tripolyphosphate (STPP) and rusk were purchased from All in All Ingredients (Dublin, Ireland). All breakfast sausage formulations were made containing pork lean meat (58%), pork back fat (20.35%), water/ice (13.45%), rusk (5.75%) and seasoning (1.45%), along with the designed mixture of ingredients (AP, CSS or TPP to a constant level of 1%) according to experimental design. Sausage batters of each formulation were prepared in three separate batches of each 1kg, and the sausages were ready from those batters to obtain the replication. Table 3-1 describes the experimental design developed by mixture design software (Design Expert v. 10, Stat-Ease Inc., Minneapolis, MN, USA) for the three variable ingredients (X_1 = STPP, X_2 = AP, X_3 = CSS) used in sausage formulations. The ingredients were mixed in a bowl using hands for about 10 minutes. Then the sausage batter was stuffed inside the collagen casing of 23 mm diameter (Select Collagen Casings, Glasgow, Scotland) using the meat mincer with sausage filler (Meat Grinder MG510, Kenwood, UK). The sausages (~10 cm in length) were then kept in a tray of height 197 mm x width 155 mm x depth 30 mm (Silverstream packaging Ltd, Cork, Ireland), wrapped using the cling film (gas permeability -2.5 [g 100µm]/ [m^2d], 300 mm x 300 M, Prowrap, Bristol, UK). The trays were assigned based on the analysis performed, and the number of sausages in the individual tray depended on its assigned techno-functional analysis. The packed trays were stored in the retail storage refrigeration unit (EXPO PT, glass door upright display cooler, Framec, Italy) at 3 – 5 °C on the day of manufacture (Day 0) for further analyses. Trays assigned for WHC, cook loss, NMR analysis, texture and compositional analysis were taken out the next day (Day 1), and respective analyses were performed. Trays assigned for a particular period of TBARS (Day 0, 3, 6 & 9) analysis were taken out on the individual days and analysis was performed.

Table 3-1: Experimental design of three components in sausage formulations

Meat Blocks	Treatment run	STTP(%)(X_1)	Apple Pomace(%)(X_2)	CSS(%)(X_3)
1	**1**	0	1	0
	2	0	1	0
	3	0	0	1
	4	0.5	0.232	0.268
	5	0.202	0.398	0.4
	6	0.202	0.398	0.4
	7	0	0	1
	8	0	0.493	0.507
2	**9**	0.256	0.681	0.063
	10	0	0.241	0.759
	11	0.329	0.484	0.187
	12	0.257	0.68	0.063
	13	0.5	0	0.5
3	**14**	0.5	0.5	0
	15	0	0.728	0.272
	16	0.257	0	0.743
	17	0.257	0	0.743
	18	0.259	0.189	0.552

Where $X_1 + X_2 + X_3 = 1\%$, $(0 \leq X_1 \geq 0.5)$; $(0 \leq X_2$ and $X_3 \geq 1)$

3.2.2 WHC and Cook Loss

Three samples from each batch per treatment were taken from the retail unit the day after the sausage production (day 1) and analysed for WHC as described by Lianji & Chen (1989) with some modifications**.** Approximately 10 g of raw sample (weight B) were weighed in the 50 ml centrifuge tube and heated in a 90 °C water bath for 10 minutes. After heating, the samples were removed from the tube and cooled to room

temperature. The samples were weighed (weight C) and wrapped with a cheesecloth in a 50 ml centrifuge tube filled with absorbent cotton wool (filled up to 1/3 part of the tube). Then the samples were centrifuged for 10 min at 204 x g (4°C) in a Sorvall Lynx 6000 centrifuge (Fischer Scientific Ireland, Dublin, Ireland), and the samples in the tube were weighed (weight A). The following equations then calculated the WHC and cook loss:

$$\text{WHC } (\%) = 1 - \frac{(B-A)}{M}\ x\ 100 \quad (1)$$

Where M was the total water content in sample meat calculated from the moisture values determined using the SmartTrac rapid fat/moisture analyser (SmartTrac 6, CEM Corporation, NC, USA) based on the AOAC methods 985.14 (1991) by microwave method.

$$\text{Cook Loss } (\%) = \frac{Initial\ weight\ (B) - Cooked\ weight\ (C)}{Initial\ weight\ (B)} x\ 100 \quad (2)$$

3.2.3 Texture Analysis

Five raw sausages (day 1) from each treatment were taken and cooked in a water bath for 20-30 minutes at 73±1 °C until the core of the sausages was cooked at 70 °C. The sausages were cooled and analysed for texture profile analysis (TPA). The sausages were cored (diam. 14 mm x ht. 20 mm) and axially compressed to 70% of their original height at a crosshead speed of 100mm/min in a two-cycle compression test using an Instron universal testing machine model 5543 (Instron (UK) Ltd., High Wycombe, UK) attached with compression anvil. Force time deformation curves were obtained using a 500 N load cell. The following attributes were recorded using the TPA: hardness (N), gumminess (N), Chewiness (N), springiness (mm) and Cohesion force ratio as described by (Bourne, 1978). The average values for five cores per treatment were recorded.

3.2.4 Colour Measurement

Instrumental Colour measurements of three sausages (day 1) per treatment were recorded using an UltraScan Pro (Hunterlab, Reston, VA, USA) dual beam xenon flash spectrometer, with a viewing port of 25.54 mm and illuminant D65, 10^{o}. The specular component was included (RSEX included), and the trap size was 9 mm. Calibration

was carried out using a light trap (L = 0) and a white standard tile (L = 100; X = 88.69; Y = 93.58; Z = 100.45), covered in the same material as the sample (transparent cling film) to eliminate any colour readings effect. The sausages were packaged into a PVC film to measure colour to maintain reading method uniformity. The colour measurements were expressed in L* (Lightness/ darkness), a* (redness/greenness) and b* (yellowness/blueness) units. The values were measured in triplicates and averaged for statistical analysis. The total colour difference (ΔE) was calculated using the formula obtained from Salgado, Fernández, Drago, & Mauri (2011).

$$\Delta E^*_{ab} = \sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2} \quad (3)$$

3.2.5 Lipid Oxidation

This analysis was performed based on the method described by Botsoglou et al. (1994) with some modifications. MilliQ water of 20 mL was added to 1.5 g of raw blended sausage and homogenised with an Ultraturrax homogeniser (Labortechnik, Staufen, Germany) at 13500 rpm for 30s. Cold trichloroacetic acid (25% TCA) of 5 mL was added, followed by gentle stirring at 4 ^{o}C for 15 min. The supernatant was obtained by centrifugation at 3500 rpm for 15 min (4 ^{o}C). A 3.5 mL of the supernatant was mixed with 1.5 mL of 0.6% 2-thiobarbituric acid with the reaction performed in a water bath at 70 ^{o}C for 30 min. The tubes were cooled, and TBARS was measured at 532 nm using UV-Vis Spectrophotometer (Shimadzu UV – 1700, Columbia, USA). A standard curve was prepared as per the procedure of (Maraschiello, Sárraga, & García Regueiro, 1999). The results were expressed as milligrams of malondialdehyde per kilogram of sausage (mg MDA/kg). Three sausage samples per batch of each formulation from days 0, 3, 6 and 9 of storage were analysed for the TBARS method.

3.2.6 Low-field field NMR transverse relaxation measurements

The NMR experiment was performed precisely as McDonnell et al. (2013) described using a Maran Ultra instrument (Oxford Instruments, Abington, Oxfordshire, UK) at a resonating frequency of 23.2 MHz. Three measurements were performed on each sample and averaged for statistical analysis.

3.2.7 Compositional Analysis

Sausage batters from all three batches of each treatment run were homogenised using the blender (Blender CH180A, Kenwood, China) for 5 minutes, and triplicates sub-samples were analysed. Moisture and fat content were determined by SmartTrac5 rapid fat/moisture analyser (SmartTrac 6, CEM Corporation, North Carolina, USA) using AOAC 985.14 (1991) and AOAC 2008.06 (2008), respectively. Protein content was analysed using the LECO Nitrogen content determiner (LECO FP628, LECO Corporation, Michigan, USA) using 6.25 as the nitrogen to the protein conversion factor. Protein analysis was based on the Dumas method and according to AOAC 992.15 (1992). Ash content was determined using a 550 °C Gellenkamp heating furnace using AOAC 920.153 (1920), and salt content (NaCl) was measured from the ash using the Bohr titration method based on AOAC 935.47 (1987). The total crude fibre content was measured using the ANKOM^{TDF} Dietary Fibre Analyser (ANKOM Technology, NY, USA) according to AOAC 991.43 (1994) enzymatic-gravimetric method**.** The values were averaged for statistical analyses.

3.2.8 Statistical Analysis

The Mixture design experiment was designed and analysed using the statistical software Design Expert (v. 10, Stat- Ease Inc., Minneapolis, MN, USA). The factor response characteristics of the ingredients were studied using an I-optimal design type with a quadratic design model for the three-ingredient mixture systems with three meat blocks. The experimental design was divided into three meat blocks. For each block, separate batches of pork loins were employed to consider potential variations derived from the different raw materials employed in each block. As per the requirement for each meat block, different numbers of pork loins were purchased. The four constraints used were (i) Phosphate STTP ($0\% \leq STTP \geq 0.5\%$); (ii) AP (X_2) ($0\% \leq X_2 \geq 1\%$); (iii) CSS (X_3) ($0\% \leq X_3 \geq 1\%$); (iv) total ingredients (CSS + AP + STPP = 1%). This design was developed to study the phosphate replacing abilities of AP and CSS. The experiment consisted of 18 runs representing the different substitution levels for STPP (e.g. 100%, 50%, 40% substitution) by the natural ingredients. To create a strong design similar to the works of Keenan et al. (2014) & Baugreet et al. (2017), the experimental design contains, with few modifications,

replications of maxima of the replacers without any phosphate percent (run 1 & 2, 3 & 7), maxima of ingredients with reduced phosphate percent (run 9 & 12, 16 & 17) and equal distribution of both ingredients with reduced phosphate percentage (run 5 & 6). The table was completed with the responses obtained after the analysis of the sausages, and the experiments were carried out in random order to eliminate the extraneous factors in the observed responses. As responses, parameters such as WHC, cook loss, texture, and proximate composition (fat, moisture, protein and ash) were assessed. After the results collection, the responses were calculated using the linear (4), quadratic (5) or Scheffe's special cubic models (6) depending on the degree of fit, predictive power and robustness of the model.

$$Y = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 \quad (4)$$

$$Y = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 \quad (5)$$

$$Y = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{123} x_1 x_2 x_3 \quad (6)$$

Where Y is the predicted dependent variable, β is the equation coefficient, and x is the proportion of pseudo components. The dependent variables were analysed, and the model was subjected to analysis of variance (ANOVA) to determine the significance ($P<0.05$), determination coefficient (R^2) and lack of fit. The software's optimisation tool subsequently analysed significant dependent variables to predict optimal sausage formulations.

3.3 Results & Discussions

3.3.1 Compositional Analysis

Mean values of moisture, fat, protein, ash, fibre and salt content of the sausages were given in Table 3-2. Each parameter was thoroughly analysed and fitted with an appropriate significant response surface model. The model of compositional analysis of moisture, protein, fat and salt (sodium chloride) contents were found to be not significantly different; which was expected since the formulation was designed to be identical (approximate target values: Moisture ~ Moisture ~65%; Fat~12.4%; Ash ~2%; Protein ~15.2%; Salt ~0.8%) but for the 1% corresponding to functional ingredients. The ash content of the sausages was fitted with a linear equation. The

model was significant with the experimental data fit (R^2 = 0.72). From Figure 3-1d, the ash content gradually increases with increased phosphorus concentration. The definition can explain that ash content represents inorganic mineral residue remaining after igniting and oxidation of organic matter (Nunes, De Oliveira Matias, & Da Silva Catalao, 2017). Thus, when added, phosphorus, an inorganic mineral, increased the ash content of the sausages.

It was found that a special cubic equation can fit the total dietary fibre content of the sausages (R^2= 0.74), and the model was significant, proving the fibre analysis model is valid. In general, meat is deficient in any dietary fibre content. However, the total dietary fibre analysis showed a small number of dietary fibres in sausages. It was highly due to the addition of AP and CSS to the formulations since both AP (78-89%) and CSS (86%) are rich in their dietary fibre content (Gemechu, 2020; Iriondo-DeHond et al., 2019). Most of the sausages' average total fibre content lies in the range of 1-3%, thus, justifying the addition of AP and CSS as potential phosphate replacers and fibre enhancers in the sausage formulations.

Table 3-2: Mean and standard error mean values of proximate compositions, LF-NMR percentage population and TBARS values of days 0, 3, 6 and 9.

Treatment	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	Fibre (%)	Salt (%)	T_{2b}% Population	T_{21}% Population	T_{22}% Population	TBARS mg MDA/kg			
										Day 0	Day 3	Day 6	Day 9
1	66.3	9.9	17.2	1.7	1.6	0.8	2.41	91.62	5.96	0.27	0.28	0.55	0.67
2	66.4	10.0	16.7	1.6	0.7	0.7	2.15	92.72	5.12	0.22	0.26	0.99	0.79
3	66.6	9.3	16.9	1.7	1.9	0.7	2.76	92.28	4.95	0.25	0.26	0.32	0.32
4	65.6	10.8	17.0	2.0	1.8	0.7	2.48	91.74	5.76	0.18	0.22	0.39	0.38
5	64.5	12.7	16.5	1.8	1.7	0.7	1.93	91.58	6.48	0.21	0.25	0.66	0.67
6	65.5	11.0	17.2	1.8	1.3	0.7	1.98	92.55	5.46	0.2	0.32	0.43	0.72
7	65.3	10.8	17.4	1.8	1.3	0.8	1.70	93.15	5.13	0.26	0.23	0.44	0.61
8	65.1	11.1	17.4	1.7	1.8	0.7	1.46	93.43	5.09	0.27	0.33	0.48	0.85
9	62.9	15.1	13.9	1.8	2.4	0.6	1.90	90.59	7.50	0.32	0.26	0.54	0.70
10	64.4	12.8	15.6	1.7	2.0	0.8	2.29	90.86	6.84	0.25	0.26	0.29	0.60
11	65.7	11.3	15.6	2.0	2.8	1.0	2.11	91.93	5.95	0.19	0.22	0.39	0.53
12	63.5	15.1	14.0	2.0	4.0	1.0	1.99	92.54	5.46	0.21	0.31	0.79	1.13
13	66.0	11.5	14.4	2.0	5.3	0.5	2.46	91.40	6.13	0.16	0.17	0.32	0.37
14	63.2	13.9	15.9	2.0	3.6	0.6	2.84	88.41	8.74	0.23	0.30	0.33	0.82
15	63.3	13.6	16.8	1.8	0.3	0.9	2.09	90.82	7.08	0.28	0.31	0.53	0.92
16	63.3	13.3	16.2	1.9	1.7	0.7	3.24	88.70	8.05	0.19	0.23	0.36	0.48
17	62.5	14.3	16.4	1.9	1.5	0.6	3.77	87.53	8.68	0.21	0.40	0.46	0.59
18	64.7	12.3	16.9	1.9	0.7	0.9	2.92	90.03	7.04	0.20	0.19	0.31	0.36
Model Significance	ns	ns	ns	**	*	ns	***	*	ns	*	ns	*	*
SEM*	0.31	0.42	0.27	0.03	0.29	0.03	0.14	0.39	0.29	0.01	0.01	0.04	0.05

Where *, ** and *** are model significance levels at $P < 0.05$; $P < 0.01$; $P < 0.001$ respectively, ns- not significant

SEM* - Standard Error of Mean

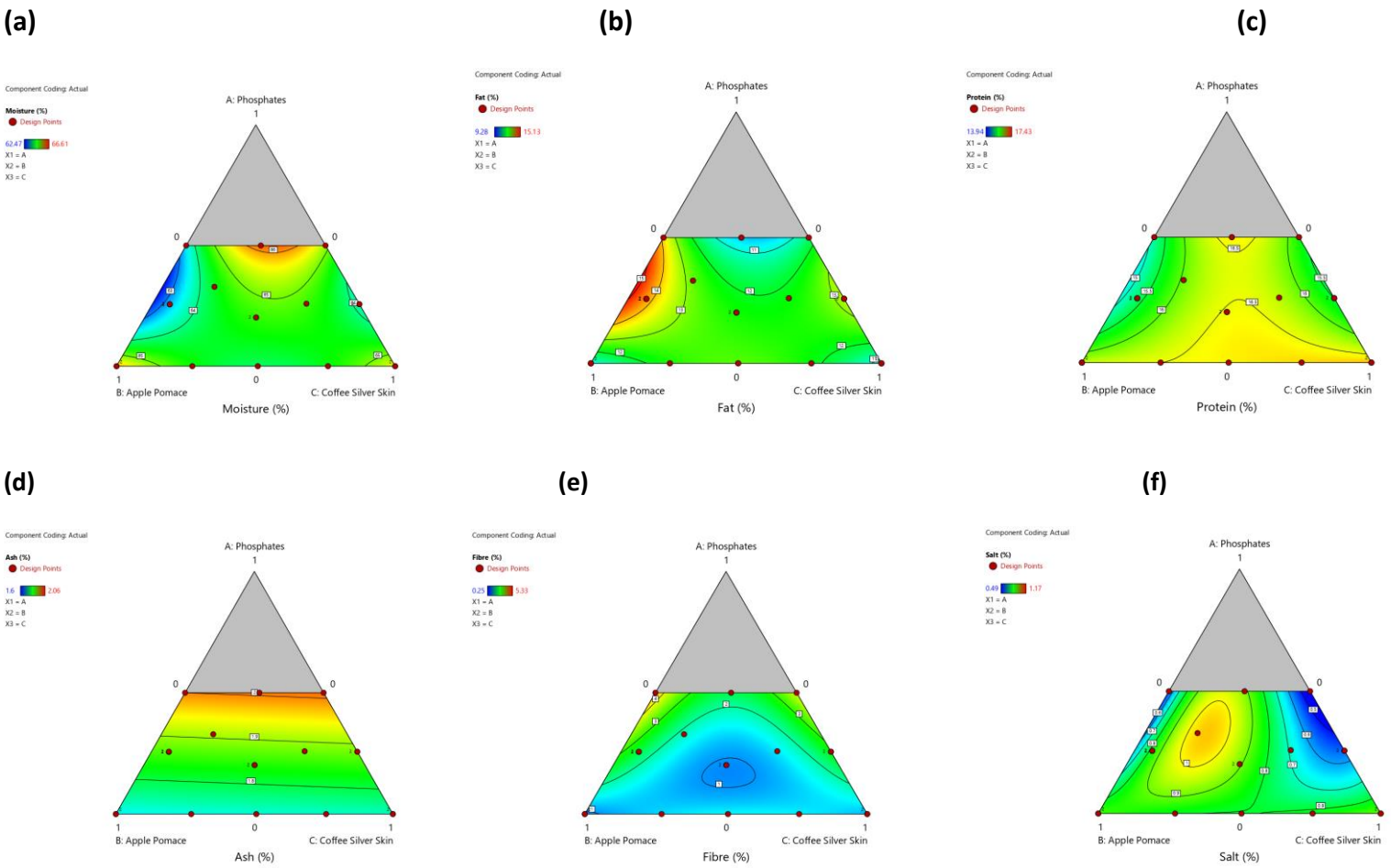


Figure 3-1: Contour plots of different proximate compositional parameters of sausages (1a) Moisture, (1b) Fat, (1c) Protein, (1d) Ash, (1e) Fibre and (1f) salt.

3.3.2 WHC & cook loss

Results of WHC and cook loss of different sausage formulations are given in Table 3-3. WHC values were fitted with a quadratic model, and the cook loss data were fitted with a linear model. The predicted model for both the WHC and cook loss was significant with a fit of experimental data (R^2 = 0.92 for WHC; R^2 = 0.74 for cook loss). The RSM model for the prediction of WHC and cook loss in terms of the actual components was determined as follows:

$$WHC = 74.18\ x_1 + 72.72\ x_2 + 73.82\ x_3 + 55.23\ x_1x_2 + 46.66\ x_1x_3 + 10.37\ x_2x_3 \quad (7)$$

$$\text{Cook loss} = -0.48\ x_1 + 12.37\ x_2 + 13.03\ x_3 \quad (8)$$

ANOVA and post-result analysis of WHC showed that linear and quadratic effects of phosphates were the most affecting factors on WHC of sausages. It was observed that the values of WHC were low (68-72%) for the sausages containing no STPP compared to the sausages containing different concentrations of the STPP (Figure 3-2a). The higher values of WHC (85-88%) were observed in the sausage formulations containing 0.5% of added STPP (run 4, 13, 14). Table 3-3 shows that the WHC of sausages decreased with the reduction in STPP concentration when compared to the one with 0.5% STPP added to them. This can be attributed that phosphates are perfect meat water binders and help improve the WHC of the meat products (Nguyen Huynh Bach Son, 2011). However, the difference in reduction of WHC of sausages with reduced STPP (run 5, 6 - 0.202%, run 9 - 0.256%, run 12, 16, 17 - 0.257%, run 18 - 0.259%) was very small when compared with the runs 4, 13 and 14. It was also noted that run 11 with 0.329% of STPP recorded higher WHC (88.6%) along with AP and CSS mixtures. It was evident from the results that the STPP has a positive interaction with the alternative ingredients. The addition of AP and CSS to the sausage formulations has a significant positive impact on the WHC. This could be explained by the synergistic contribution of different AP and CSS mixture concentrations in their formulations. Despite the reduction in STPP concentration, adding AP and CSS improved the WHC (Ballesteros, Teixeira, & Mussatto, 2014; Younis & Ahmad, 2015) because of their high dietary fibre content.

Although in the opposite direction, a similar trend of WHC values was observed in the cook loss data of the sausages. Sausages containing reduced levels of STPP recorded lower cook loss values (5.7-13.4%) than those without STPP (9.3 -18%). This proved that both the ingredients AP and CSS could not fully counteract the absence of STPP in terms of WHC and cook loss. Thus, proving the complete replacement of STPP in sausages is challenging.

However, lower cook loss value (5.6%) was recorded for the sausage containing 0.329% of STPP with 0.484% AP & 0.187% CSS in them. It was also noted that the cook loss values of formulations containing higher STPP levels (0.5%) were nearly the same as those containing lower level STPP, thus implying the addition of AP and CSS powders to the formulations compensated for the increased cook loss due to the reduction in STPP concentration. This can be explained by their ability to decrease the cooking loss in the meat products (Younis & Ahmad, 2015; Martuscelli, Esposito, and Mastrocola, 2021b), thus making these ingredients potential phosphate replacers in meat products.

(a) **(b)**

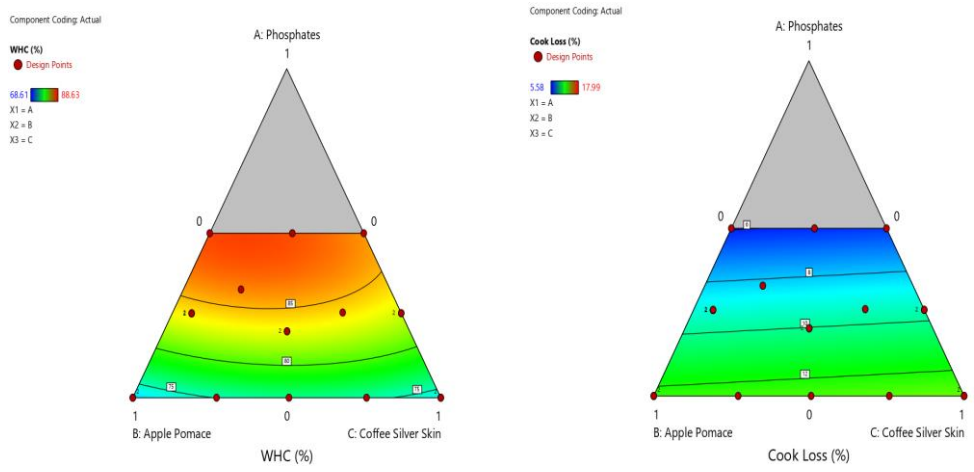


Figure 3-2: Contour plots of water holding capacity (a), cook loss (b)

Table 3-3: Mean and standard error mean values of water holding capacity, cook loss, colour and textural parameters of sausages.

Treatment	WHC (%)	Cook loss (%)	Colour				Hardness(N)	Chewiness(J)	Cohesive force	Gumminess(N)	Springiness(mm)
			L*	a*	b*	ΔE					
1	69.6	16.1	63.4	6.6	20.1	1.74	21.6	23.1	0.7	3.8	6.1
2	68.6	18.0	61.9	6.9	20.1	0.68	19.0	18.4	0.7	3.4	5.3
3	69.2	17.9	57.6	6.6	18.6	4.48	24.5	25.9	0.7	4.7	5.5
4	85.2	8.3	61.9	7.4	19.6	Control	37.4	95.5	0.6	11.4	8.3
5	80.9	11.0	61.1	7.0	19.1	1.01	29.1	46.2	0.8	6.4	7.9
6	77.7	13.4	59.2	7.3	19.2	2.73	24.2	45.4	0.8	5.9	7.6
7	72.7	15.9	57.5	6.5	17.8	4.81	19.2	18.5	0.7	3.6	5.1
8	71.6	16.7	58.9	6.1	17.4	3.97	16.9	14.5	0.7	2.9	5.0
9	87.2	5.7	64.5	7.7	19.7	2.66	23.0	57.8	0.8	7.8	7.1
10	77.5	9.3	60.3	7.0	19.0	1.74	14.0	13.5	0.7	2.8	4.8
11	88.6	5.6	63.8	8.2	19.6	2.09	31.8	77.9	0.5	10.7	7.4
12	87.5	5.9	65.6	7.5	20.2	3.71	20.7	51.8	0.8	6.5	7.9
13	88.2	6.3	62.9	7.4	20.2	1.18	22.7	43.3	0.7	6.1	7.0
14	86.5	5.8	66.7	6.3	19.5	4.97	53.6	148.5	0.6	17.3	8.6
15	76.9	11.3	62.2	6.1	19.2	1.36	18.2	15.2	0.8	3.1	4.9
16	84.2	8.1	59.7	6.1	18.1	2.91	41.5	104.0	0.6	12.6	8.3
17	81.1	10.0	61.0	6.4	19.1	1.40	31.3	60.7	0.8	8.3	7.4
18	86.7	7.3	59.8	5.9	18.1	2.93	46.3	118.4	0.6	14.3	8.3
Model Significance	***	***	***	**	***		***	**	ns	**	***
SEM*	1.70	1.07	0.62	0.15	0.2		2.58	9.42	0.02	1.02	0.32

Where *, ** and *** are model significance levels at $P < 0.05$; $P < 0.01$; $P < 0.001$ respectively, ns- not significant

SEM* - Standard Error of Mean

3.3.3 LF-NMR Measurements

The increase in WHC of sausages due to the addition of AP and CSS can be explained in detail using the low field NMR T_2 relaxation data of the samples. The relaxation data can be used to understand the water mobility and distribution in the meat matrix. It is reported that there is a high correlation between the relaxation data T_2 and WHC traits of the meat matrix (Bertram, Purslow, & Andersen, 2002).

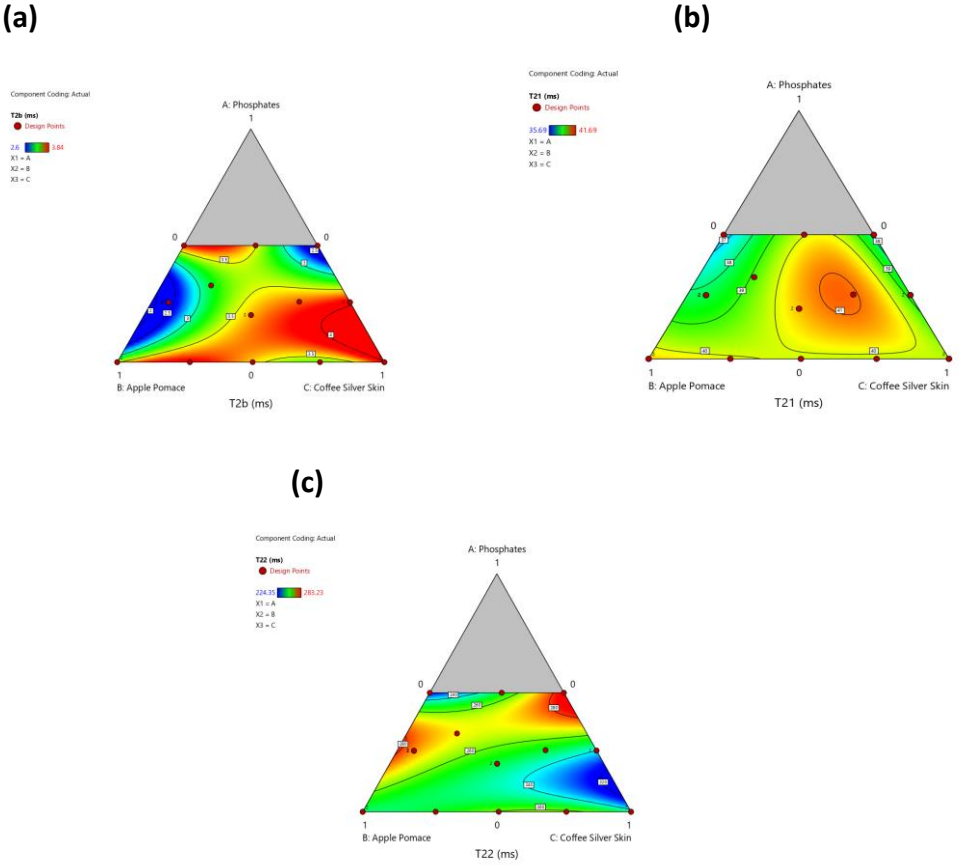


Figure 3-3: Contour plots of LF-NMR values of sausages where (a) T_{2b}, (b) T_{21} and (c) T_{22}.

The literature reported (Bertram, Andersen, & Karlsson, 2001) that meat samples' NMR relaxation time distribution graph contains three components distributed at different time intervals when fitted with exponential fitting. The first component, a minor one, is distributed between 1- 10 ms, the second one, a significant component, is distributed between 10 -100ms and the final part is distributed between 100 – 1000 ms. It is understood that the relaxation time obtained through the low field NMR analysis on meat samples can be used to demonstrate the water mobility, and

the area under the curve can indicate the amount of water present within the meat matrix. Thus, the T_{2b} relaxation time (1 -10 ms peak value) corresponds to the bound water that is bound to proteins, the T_{21} relaxation time (40 - 60 ms peak value) corresponds to the myofibrillar water, and the relaxation time T_{22} relaxation time (150 – 400 ms peak value) corresponds to the free water present outside the myofibril lattice and muscle cell which can be considered as a potential drip loss (Bertram et al., 2001).

In this study, the three peaks obtained from the multi-exponential fitting of T_2 relaxation data resembled the observations mentioned by Bertram et al. (2001). Table 3-2 contains the percentage population distribution of different sausage formulations of T2b, T21, and T22. Response surface method analysis shows that there was a significant change in the T_{2b} and T_{21} values (Figure 3-3), and there were not any significant changes in T_{22} relaxation data values implying that the addition of ingredients AP and CSS have much positive effect on controlling the drip loss despite the reduction in phosphate concentration. The T_{2b} and T_{21} values are fitted with cubic and special cubic equations (R^2 = 0.96 and R^2 = 0.87). The protein-bound water molecules (T_{2b}) do not vary with mechanical stress and changes in the meat matrix (Bertram et al., 2001; McDonnell et al., 2013). However, there was a significant change in the T2b values in this study due to the addition of AP and CSS. It was observed from the results that the values of T_{21} and T_{2b} of all the sausages are very much in range with each other within the blocks, which may be due to the different meat used in each one of them. It was observed that the ingredients did not affect the T_{22} distribution time and its population percentages within the meat blocks. The population percentages data of all three curves of all 18 treatments showed that water distribution was almost similar within the meat blocks, and slight variation in the area and population distribution was observed in terms of the concentration of the ingredients. It was observed from the study that the NMR curve area and water distribution of the treatment run 1, 2, 3, 7, 10 and 15 (runs without STPP) had almost similar or higher T_{21} water distribution when compared with the other treatment runs with STPP in them. A similar trend in the opposite was observed with T_{22} values where the treatment runs without STPP (1, 2, 3, 7, 10 and 15) had lesser water

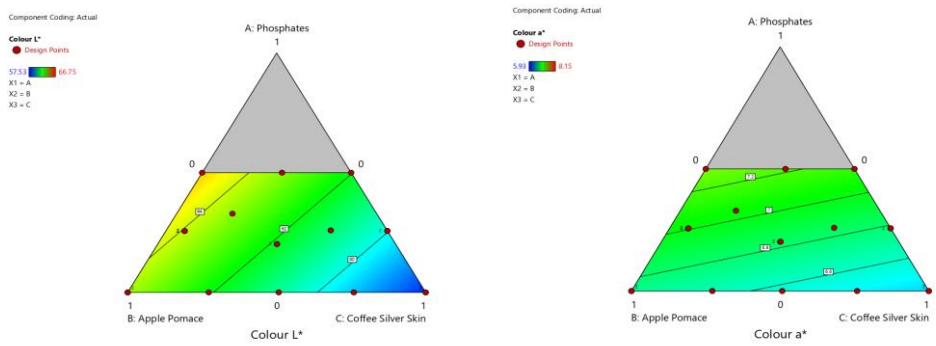
distribution and lesser T_{22} area when compared with the ones with STPP in them, proving the above statement that the addition of AP and CSS has a positive effect on controlling the drip loss despite the reduction in phosphate concentration.

3.3.4 Instrumental Colour

Experimental data for the colour of the sausages is shown in Table 3-3. The responses observed for all three parameters of colour measurements L*, a* and b* were significantly different in the response surface method. The linear model predicted L* to be significant with a proper experimental fit (R^2 = 0.85). It was noted from Figure 3-4a that the increased addition of CSS to formulations decreased the L* values, which was in agreement with the experimental analysis of Martuscelli, Esposito, and Mastrocola (2021b), where the L* of CSS decreased with the increased concentrations. It was also seen that formulations run 9, 12 and 14 recorded higher L* values than the other formulations indicating the interaction effect of STPP with AP in increasing the L* values. Regarding a* values of the sausages, it is clear from Figure 3-4b that increased STPP concentration increased a* values up to a maximum of 7.30 and, in other terms, increased addition of AP and CSS to the sausages reduced a* values of the meat. The values of a* were fitted with a linear model with significant data of R^2 = 0.46. Including CSS in the formulations decreased the b* values compared with sausages containing AP and STPP (Figure 3-4c). The b* values dropped from a high of 19.67 and 20.13 (full STPP and full AP, respectively) to a low of 17.8 (full CSS) with increased concentration of CSS. The model for b* values were fitted with a significant linear model with an experimental fit of only R^2 = 0.53 between the predicted and adjusted values. In general, Younis and Ahmad (2015) reported that the overall increased values of L*, a* and b*could be observed due to the inclusion of AP in buffalo meat sausages. This contradicts the data obtained, which may be due to the addition of CSS alongside AP, which caused a reduction in the colour parameters. On observing the ΔE (measured using Eq. 3) of the sausages compared with the one from run 4 (containing maximum STPP and equal AP: CSS) in Table 3-3, it was observed that there were no much visible differences ($0<\Delta E<2$) for most of sausages trial runs (1, 2, 5, 10, 11, 13, 15 and 17). However, a clear colour difference ($\Delta E>2$) for inexperienced observers was observed in trial runs (3, 6, 7, 8, 9,

12, 14, 16 and 18). It is understood that no differences in colour between the samples would be observed if 0<ΔE<2, and a clear colour difference could be observed if ΔE>2 between two samples (Álvarez, Drummond, & Mullen, 2018). Comparing the above results of ΔE, the same trial run combination 5 and 6 does not fall under the same category. A similar trend was observed for the other trial run combinations 16 and 17, where the trial runs 5 and 17 had no visible difference compared with a trial run 4. In contrast, trial runs 6 and 16 showed a clear colour difference compared with a trial run 4. The colour difference values for 16 and 17 are negligible, and there was less observed visual difference between the sausage formulations disregarding the experimental data. It was also observed from the Tables 3-3 that the formulation runs 3 and 7 with higher CSS concentration (CSS -1 %) showed a more significant colour difference (ΔE>4) when compared with the trial runs 1 and 2, where the AP concentration is higher (AP= 1%; ΔE<2). Thus, the complete replacement of STPP with AP and CSS could pose a challenge since the change in colour difference could affect the consumer preference for the products.

(a) **(b)**



(c)

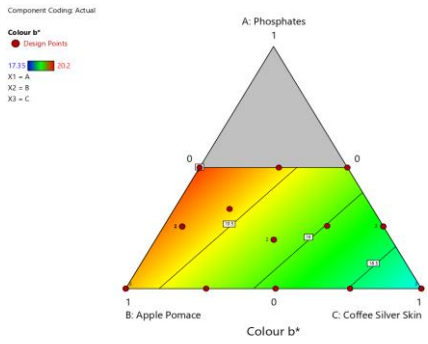


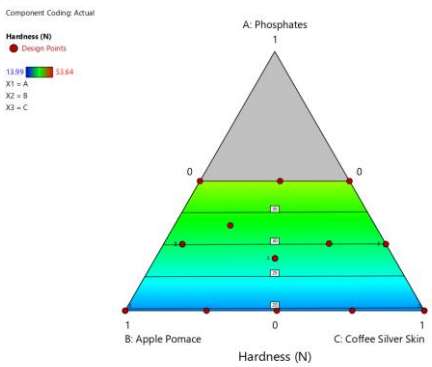
Figure 3-4: Contour plots of instrumental colour parameters L* (a), a* (b) & b* (c).

3.3.5 Textural Properties

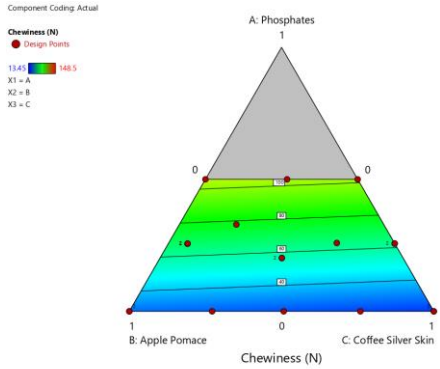
The texture of processed meat products is an essential parameter from the customer's perspective. In post-research analysis, similar to Keenan et al. (2014), in fat replaced sausages with added inulin, four textural properties were significantly different except cohesive force. From the results, the reduction of STPP in sausages composition significantly reduced the hardness, chewiness, gumminess, and springiness values compared with the sausages containing higher STPP content. This stable textural property with increased addition of STPP could be explained by its ability to increase the ion strength of meat to extract the salt-soluble proteins (Choe et al., 2018). Mean values of the textural parameters were found in Table 3-3. The hardness of sausages fitted with the linear model was significant with an experimental data fit (R^2 = 0.64). Figure 3-5a shows that there is a significant decrease in the hardness of sausages with an increase in AP and CSS (Hardness range - 13- 24 N) concentrations when compared with the sausages with high STPP content (Hardness range (37 -54 N). Also, from the observed data in Table 3-3, three different meat blocks seemed to affect sausages' overall textural data, especially block 3. Hence, an increase in hardness values can be attributed to the other meat blocks used and the different inherent textural properties of the meat samples employed rather than an impact of the different formulations (Wang, Xu, & Zhou, 2009). A similar trend was noticed in the chewiness of the sausages. The chewiness was significant when fitted with the linear model with R^2 = 0.68. It was observed from Figure 3-5b that chewiness decreased with reduced STPP levels. Gumminess, in turn, was fitted with a cubic model and was found to be significant with a good fit of R^2 = 0.94. From the experimental data (Table 3-3), it was evident that chewiness and gumminess values were higher for sausages with higher STPP content, and reduced values were recorded for sausages without any added STPP but with added ingredients in them. Similarly, springiness was significant when fitted with the quadratic model with the agreement of experimental data (R^2 = 0.93). As with other textural parameters, the springiness values of the sausages were reduced with the addition of AP and CSS. This reduction in springiness could be explained by the protein-water or protein-protein gelation networks formed due to adding fibres to the meat products (Han and Bertram, 2017). Thus, the complete replacement of STPP

with AP and CSS could be challenging since they could result in a softer product texture, influencing consumer preference for the product.

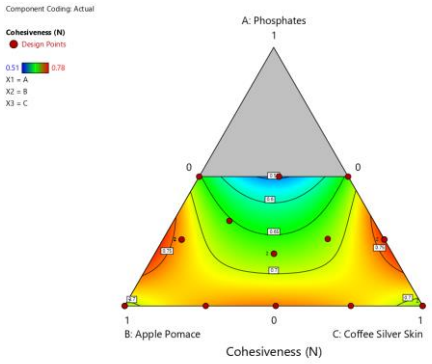
(a)



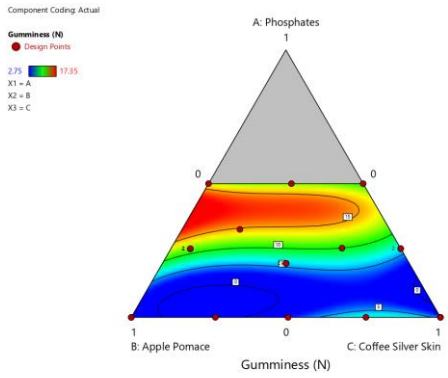
(b)



(c)



(d)



(e)

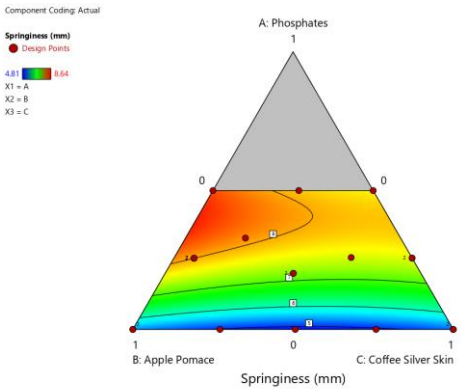


Figure 3-5: Contour plots for the textural parameters of formulations where (a) Hardness, (b) Chewiness, (c) Cohesiveness, (d) Gumminess and (e) Springiness.

3.3.6 Lipid Oxidation

Lipid oxidation analysis is based on the detection of malondialdehyde (MDA) formed as a by-product of peroxidation of polyunsaturated fatty acids and esters, associated with off-flavours and off-aromas in meat products (Maraschiello, Sárraga, & García Regueiro, 1999; Pérez-Andrés et al., 2020). Table 3-2 shows the TBARS results of day 0, 3, 6 and 9 sausages samples stored at 4 ^{o}C. Although not considered antioxidants, phosphates added to meat products inhibit lipid oxidation by chelating the metal ions and removing the catalysts responsible for lipid oxidation (Choe et al., 2018). It can be observed that decreasing the concentration of STPP in the sausages resulted in a slight increase in TBARS values. However, sausage formulations containing no phosphates recorded TBARS value (day 9) very much in the range of those containing different levels of phosphates.

All the lipid oxidation values were analysed using Mixture design software. Day 0 and 6 analysis was fitted with linear equations (R^2 =0.47 and R^2= 0.46 respectively) and are highly significant. Similarly, day 9 was fitted with a special cubic model (R^2 = 0.76) and is statistically significant with their experimental data. The values of day 3 were not significantly different from each other, implying none of the ingredients impacted TBARs value for day 3. Table 3-2 shows that the TBARS MDA values of days 0 and 3 are very similar to each other and fall within the range of 0.15 – 0.30 mg MDA/kg. The difference in lipid oxidation values can be observed from the TBARS analysis of days 6 and 9. However, the day 9 values of all the sausages lie within the threshold limit of 2-2.5 mg MDA/ kg, where no rancid flavours were observed in meat and meat products (Campo et al., 2006; Zhang et al., 2019).

In Figure 3-6d, samples containing a higher concentration of AP (run 1, 2, 8, 9, 11, 12, 14 and 15) recorded comparatively higher MDA content on day 9 than the other products. Considering the CSS application, the sausages containing a high concentration of CSS (run 3, 7, 10, 13, 16 and 18) showed a positive response against the MDA content. Their low values can be attributed to CSS being an excellent antioxidant (Iriondo-DeHond et al., 2019). This may indicate that reducing the phosphates on TBARS values is negligible, and they are well counter- interacted by

adding AP and CSS to the mixture. The antioxidant property of the AP and CSS can inhibit lipid oxidation in phosphate-replaced sausages.

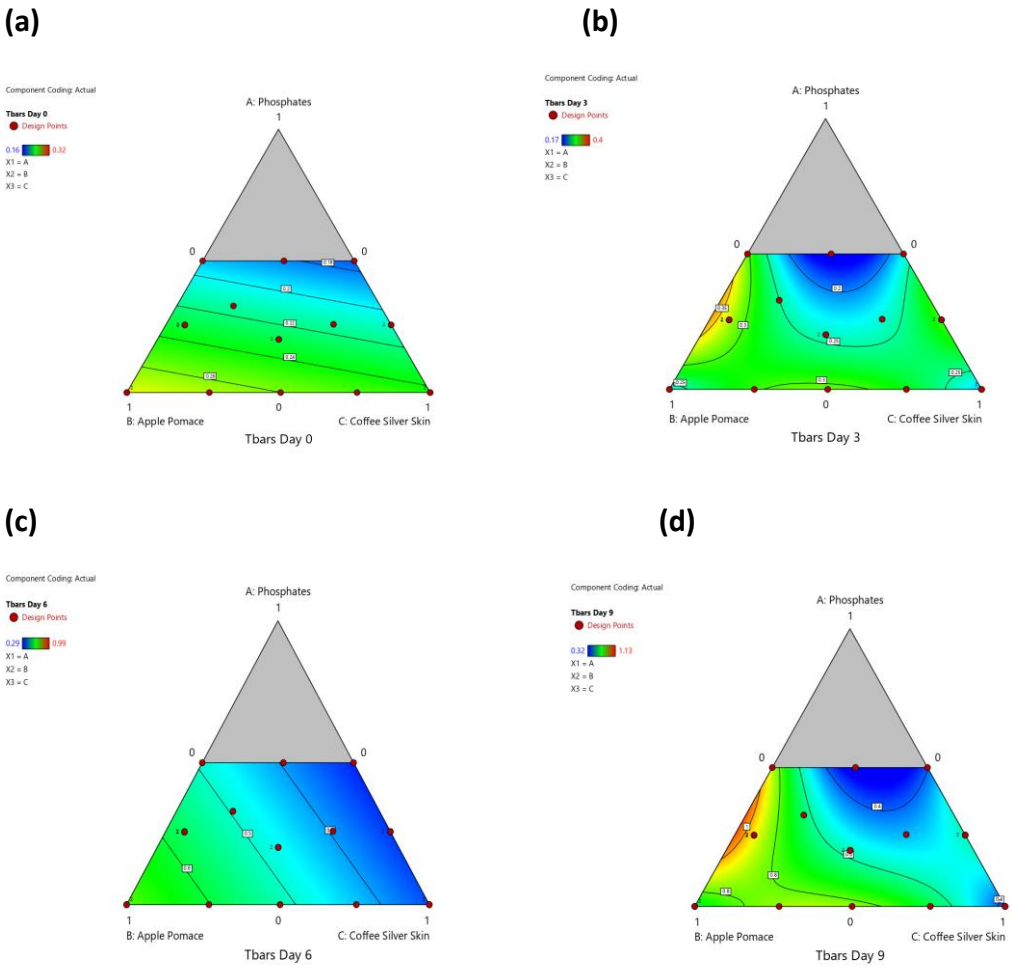


Figure 3-6: Contour plots of TBARS values of day 0 (a), 3 (b), 6 (c) and 9 (d).

3.3.7 Optimisation of sausage formulation mixtures

Numerical optimisation was performed using the Design expert optimisation tool to obtain the satisfactory optimum level of independent variables that helps improve the products' overall quality. This study used the Desirability response method to obtain the optimised concentration of independent variables (Keenan et al., 2014). The desirability function suggests the desired value range for each response which varies from 0 to 1. The objective was to maximise or minimise particular response(s) to obtain the desired product range. The results observed that the complete replacement of STPP in Irish breakfast sausages was difficult since the complete replacement could reduce the product quality. Thus, in this study, the addition of

phosphates level was minimised to the range of 0 to 0.2%, with AP and CSS addition maximised from 0 to 1%, with the total amount of all three ingredients employed being set at a 1% goal. The dependent response WHC was maximised, whereas cook loss and TBARS values of days 0, 3, 6 and 9 were minimised. These specific constraints were applied to highlight the main functionality of phosphates in meat products. The solutions obtained will focus on reducing the phosphate content without quality reduction. In other words, it was because of their closer association with the phosphates. The software predicted three possible optimised solutions with reduced phosphate levels that could improve the desired product quality. The first and second formulations had 0.2% phosphates, and the third one had 0.06 % of added phosphates. The three formulations mixture contents (STPP: AP: CSS) are (i) 0.2:0.22:0.58%, (ii) 0.2:0:0.8%, and (iii) 0.06:0.94:0%. The overall desirability of the three formulations is 0.67, 0.64 and 0.45, respectively. The predicted values of the dependent variables for the three formulations can be found in Table 3-4, which contain maximum WHC and lower cook loss values. The experimental validation of the three formulation mixtures was conducted in two trials to confirm the model's reliability. The obtained experimental WHC and cook loss values of the three experimental models are as follows: (i) 89.5±1.1 and 4.7±0.7, (ii) 90.2±0.5 and 4.5±0.4 and (iii) 87.3±2.5 and 5.7±0.3. It was noted that the obtained experimental values for WHC for three formulations were higher than the expected values. Similarly, the obtained cook loss was lower than the expected values. This difference in the expected and obtained values can be explained by the property of meat samples purchased, which could have affected the WHC properties of the final sausage product. However, it was found that there was a constantly increasing trend in obtained experimental WHC values when compared with the expected values of the three mixture formulations. All other measured parameter values were very much in order with the expected values.

Table 3-4: Optimised solutions and their predicted response obtained from Mixture design for the applied conditions

Variables	Mixture 1	Mixture 2	Mixture 3
Independent factors			
STPP(x_1)	0.2	0.2	0.06
AP(x_2)	0.22	0	0.94
CSS(x_3)	0.58	0.8	0
Total($x_1 + x_2 + x_3$)	1	1	1
Predicted optimised values with standard errors			
WHC (%)	82.8 ± 0.7	81.4 ± 0.9	75.5 ± 1.0
Cooking loss (%)	10.2 ± 0.4	10.3 ± 0.6	11.7 ± 0.7
Hardness (N)	27.7 ± 1.6	27.7 ± 2.2	21.8 ± 2.9
Chewiness (J)	47.5 ± 14.6	10.1 ± 26.7	17.1 ± 17.6
Gumminess	6.3 ± 1.6	2.5 ± 2.9	2.4 ± 1.9
Springiness (%)	7.2 ± 0.2	7.3 ± 0.2	6.1 ± 0.3
L*	60.8 ± 1.0	59.6 ± 0.4	63.8 ± 0.5
a*	7.3 ± 0.2	7.9 ± 0.4	6.7 ± 0.2
b*	18.9 ± 0.6	18.6 ± 0.2	19.7 ± 0.3
TBARS day 3 (mg MDA/kg)	0.24 ± 0.02	0.29 ± 0.03	0.27 ± 0.03
TBARS day 9 (mg MDA/kg)	0.51 ± 0.07	0.56 ± 0.09	0.80 ± 0.07
Protein (%)	16.4 ± 0.2	15.6 ± 0.3	15.9 ± 0.3
Fat (%)	12.3 ± 0.5	13.1 ± 0.7	12.2 ± 0.7
Ash (%)	1.8 ± 0.0	1.9 ± 0.03	1.7 ± 0.0
Moisture (%)	64.7 ± 0.4	63.9 ± 0.5	64.9 ± 0.5
Salt (%)	0.7 ± 0.1	0.6 ± 0.1	0.8 ± 0.1
Fibre (%)	1.2 ± 0.3	2.4 ± 0.4	1.4 ± 0.4
Desirability	**0.68**	**0.64**	**0.44**

Table 3-5: NMR time delays and related areas of the relaxation curves

Treatment	T_{2b}	T_{21}	T_{22}	T_{2b} area	T_{21} area	T_{22} area
1	3.18	39.07	252.33	9149.777	347365	22600.76
2	3.26	39.60	252.08	8127.477	349984.9	19330.16
3	3.78	39.09	242.72	10721.59	358305.9	19242.1
4	3.39	39.07	252.20	8923.517	328944.6	20688.02
5	3.32	39.58	252.08	7482.2	353895.4	25053.31
6	3.41	39.58	258.69	7415.32	346055.9	20438.82
7	3.65	38.57	258.69	6356.55	346654.7	19120.04
8	3.34	38.57	265.43	5555.103	353433.1	19261.04
9	2.86	39.58	258.69	5445.8	258682.6	21421.21
10	3.84	41.69	256.82	9950.953	393424.4	29617.62
11	3.62	40.62	265.56	8747.687	380868.8	24679.89
12	2.88	41.14	279.58	7961.397	369942.1	21849.88
13	2.60	39.58	283.23	10014.44	371791	24938.03
14	3.52	35.69	224.35	10985.71	341537.1	33774.83
15	3.66	39.58	252.08	8605.783	372283.5	29023.46
16	3.62	37.58	236.24	12621.27	344789.5	31297.41
17	3.75	38.07	242.47	14691.22	340406.5	33793.38
18	3.43	40.10	252.08	11772.14	362955.9	28419.53
Model Significance	***	*	ns			
SEM*	0.08	0.31	3.29			

Where *, ** and *** are model significance levels at $P < 0.05$; $P < 0.01$; $P < 0.001$ respectively, ns- not significant

SEM* - Standard Error of Mean

Table 3-6: Significant factors affecting responses, R2, R2 adjusted, R2 predicted and Lack of fitness of the model

	Significant Factors	R^2	R^2 adjusted	R^2 predicted	Lack of fitness
WHC	A, B, C, AB, AC	0.9278	0.8917	0.6709	0.745
Cooking loss	B, C	0.7492	0.7106	0.4884	0.522
L*	A, B, C	0.8531	0.8305	0.7228	0.534
a*	B, C, BC, BC (B-C)	0.9172	0.7931	-2.9503	0.701
b*	A, B, C	0.5302	0.4579	0.1987	0.218
Hardness	A, B, C	0.6430	0.5881	0.2316	0.266
Chewiness	BC(B-C)	0.9448	0.8620	0.5299	0.948
Gumminess	B, C, AB (A-B)	0.9441	0.8602	-0.7433	0.744
Springiness	B, C, AB, AC	0.9332	0.8998	0.7845	0.644
Cohesive force	A, B, C, AB, AC, ABC. AB (A-B), AC (A-C)	0.8541	0.6354	-6.7272	0.803
TBARS day 0	A, B, C	0.4718	0.3905	0.0098	0.101
TBARS day 3	B, C, AB, ABC	0.5044	0.1741	-0.7724	0.900
TBARS day 6	B, C	0.4690	0.3873	-0.0320	0.849
TBARS day 9	B, C, AB, BC, ABC	0.7634	0.6057	0.1555	0.287
Protein	A, B, C, AB, AC, ABC	0.6350	0.3917	-0.5806	0.333
Fat	B, C, AB, AC	0.5673	0.2788	-1.1346	0.315
Ash	A, B, C	0.7217	0.6788	0.4715	0.248
Moisture	A, B, C, AB, AC, ABC	0.5771	0.2952	-1.5288	0.283
Salt	B, C	0.6569	0.2648	-2.2967	0.004
Fibre	ABC	0.7467	0.5778	-0.6015	0.125
T_{2b}	B, C, BC (B-C)	0.9695	0.9239	-5.9470	0.279
T_{21}	A, B, C	0.8754	0.7330	-0.2840	0.061
T_{22}	B, C	0.7151	0.2879	-5.3354	0.168

A: Phosphate STPP; B: Apple Pomace; C: Coffee Silverskin.

Table 3-7: Regression Coefficient for the studied responses concerning the constraints

	A	B	C	AB	AC	BC	ABC	AB (A-B)	AC (A-C)	BC (B-C)
WHC	74.19	72.73	73.82	55.23	46.67					
Cooking loss		12.37	13.03							
L*	66.15	63.65	57.92							
a*		6.97	6.72	-2.06						8.78
b*	20.35	19.68	18.11							
Hardness	60.12	19.7	19.55							
Chewiness										-426.23
Gumminess		3.52	4.04					409.5		
Springiness		5.38	5.22	19.65	17.41					
Cohesive force	14.94	0.74	0.76	-28.92	-28.57		37.42	-20.75	-18.70	
TBARS day 0	0.10	0.28	0.24							
TBARS day 3		0.23	0.21	1.51			-4.27			
TBARS day 6		0.70	0.34							
TBARS day 9		0.67	0.36	4.58		1.32	-13.68			
Protein	20.61	16.37	16.76	-13.40	-11.82		35.78			
Fat		10.85	10.75	42.14	33.32					
Ash	2.28	1.72	1.74							
Moisture	77.17	65.88	65.57	-32.25	-24.73		70.73			
Salt		0.88	0.90							
Fibre							-59.80			
T_{2b}		3.45	3.95							4.47
T_{21}	35.62	40.48	39.85							
T_{22}		247.53	245.92							

A: Phosphate STPP; B: Apple Pomace; C: Coffee Silverskin.

3.4 Conclusion

The response surface methodology study of phosphate replacing ability of AP and CSS was well established using a mixed design approach using the Design-Expert software. The method helped reduce the number of experiments, studying several sausage formulations with reduced phosphate concentration and the impact of different ingredient combinations on the various physicochemical properties of the sausage products. Analysis of WHC and cook loss, major functionalities influenced by STPP, showed that including AP and CSS enhanced the WHC (88.6%) and reduced the cook loss (5.6%) in the formulation with reduced STPP concentration (0.33%) when compared with the likes of formulation with higher STPP concentration (0.5%). However, as a limitation of the study, the complete replacement of STPP resulted in reduced WHC, increased cook loss values and decreased textural properties. Thus, the mathematical models generated to describe the impact of the different formulations were used for the formulation optimisation; optimised recipes significantly reduced the STPP concentration (down to 0.06% -0.2%) regarding the control (0.5%) with an acceptable desirability level (0.44-0.68) where the formulations either meets or exceed the reporting requirements in reducing the phosphates. Adding AP and CSS to Irish breakfast sausages could attract health-minded consumers as they can minimise phosphate-related health problems.

Chapter 4. A comprehensive study on the characterisation properties of power ultrasound-treated apple pomace powder and coffee silverskin powder

Overview

This study investigates the effect of power ultrasound (US) treatment (250 W, 20 kHz) at different time intervals (15 and 30 mins) on the techno-functional properties of the ingredients apple pomace (AP) and coffee silverskin (CSS). The study's main aim is to focus on characterising functional properties important for ingredient application in meat products. The ingredients were analysed for proximate composition, fibre content, water absorption capacity (WAC), oil absorption capacity (OAC), colour evaluation, particle size and rheological properties. The following paper has been published in the journal - European Food Research and Technology, from this chapter:

- **Thangavelu, K. P.**, Tiwari, B. K., Kerry, J. P., & Álvarez, C. (2022). A comprehensive study on the characterisation properties of power ultrasound-treated apple pomace powder and coffee silverskin powder. *European Food Research and Technology, 248*, 1-11.

4.1 Introduction

Consumer demands for high nutrition, enhanced quality, and safer food products have led food industries to try various new alternative food processing techniques that showed some effectiveness in improving the product attributes. Therefore, developing new food products and innovative food processing techniques has been one of the most common global challenges that food scientists and food industries have faced for decades. One such innovative approach is the application of "power ultrasound (US)" in the food processing industry. The US can be employed for solid, liquid, dispersions and gaseous media. US treatment is majorly used to accelerate mass transfer, microbial and enzyme activation or inactivation (depending on processing parameters and nature of the enzyme to be treated), drying and extraction, induction of oxidation/reduction reactions, extraction of proteins and other valuable molecules (Arzeni et al., 2012; Ünver, 2016). The modification of US on food products can be explained by cavitation, heating, vigorous agitation, shear stresses and turbulence phenomena (Ashokkumar et al., 2008; O'Donnell, Tiwari, Bourke, & Cullen, 2010). The US can alter both the physical and chemical properties of food products (Hu et al., 2013). Recently, food industries have shown a massive interest in employing the US as a novel processing technology to improve the quality and safety of food products (Chandrapala, Martin, Zisu, Kentish, & Ashokkumar, 2012; Téllez-Morales, Hernández-Santo, & Rodríguez-Miranda, 2020).

Studies suggest that US treatment of food ingredients can improve their techno-functional properties and also assists in their effective distribution into food matrices, even on complicated matrices like processed meat (Alarcon-Rojo et al., 2019), where sodium chloride diffusion within products was demonstrated to be enhanced after treatment (Inguglia, Zhang, Burgess, Kerry, & Tiwari, 2018). US technology has been reported to improve water retention and oil absorption properties in meat and dairy ingredients, such as proteins, starches and lipids (Téllez-Morales et al., 2020). Jamalabadi, Saremnezhad, Bahrami, and Jafari (2019) studied the effect of the US on the techno-functional properties of wheat starch suspensions. Their studies showed improvements in starch solubility and oil absorption properties when treated with a continuous sonication of 200 W, 24 kHz (100% amplitude) for

15 and 30 minutes using the probe with a vibrating tip of a diameter of 8mm. Results of improved techno-functional properties were obtained in various studies involving US treatments of food ingredients, such as; potato, wheat, corn and rice starch (Sujka & Jamroz, 2013), proteins and polysaccharides (Köhn et al., 2016), jackfruit seed protein isolate (Resendiz-Vazquez et al., 2017), and myofibrillar proteins (Amiri, Sharifian, & Soltanizadeh, 2018).

In recent decades, studies have been conducted to develop new functional food ingredients using US technology to improve the quality of food products (Téllez-Morales et al., 2020). Additionally, consumer interest in clean label ingredients, which are natural/organic and/or non-artificial/non-synthetic, has driven food industries to research potential uses for novel, natural and under-utilised food ingredients. As mentioned above, reports (Köhn et al., 2016; Resendiz-Vazquez et al., 2017; Amiri, Sharifian, & Soltanizadeh, 2018) have shown the improvement of the meat products related to techno-functional properties such as water-holding and oil holding abilities, gelling properties and rheological characteristics, thereby making them suitable functional meat ingredients. Dietary fibre is a vital ingredient widely studied in functional meat products due to its techno-functional and medicinal applications. Dietary fibres obtained from phyto-sources offer various techno-functional benefits that improve the technological qualities of food products (Yegin et al., 2020). Studies show that fibre inclusion in meat products improves techno-functional properties, such as; swelling ability, water holding and oil holding ability, gel-forming ability, etc. (Lamsal & Faubion, 2009; Petracci, Bianchi, Mudalal, & Cavani, 2013). It is noted that the inclusion of fibre in the diet can reduce colon cancer, diabetes, obesity, and cardiovascular diseases. Thus, dietary fibres from various food sources are recommended to minimise various chronic diseases within the human body (Sah, Vasiljevic, McKechnie, & Donkor, 2016). However, these dietary fibres are not easily employed in meat products because of their carbohydrate-based polymer structure, making digestion difficult for humans (Elleuch et al., 2011) and negatively impacts functionality, thereby limiting their potential for use by the processed meat industry. Therefore, dietary fibre sources require many physical and chemical modifications to improve their techno-functional

and digestive properties. Application of the US can effectively assist in modifying the carbohydrate polymer structure (Fan et al., 2020), and previous studies have also shown that the US improves the water and oil holding capacity and solubility of the dietary fibre content (Moczkowska, Karp, Niu, & Kurek, 2019; Zhang et al., 2017). However, there is limited reporting on the impact that the US has on the techno-functional properties of dietary fibres (Téllez-Morales et al., 2020).

Apple pomace (AP) powder and coffee silverskin (CSS) powder are fibre-rich food co-products and are the ingredients of interest in this research study. AP and CSS are obtained as under-utilised co-products from apple juice and coffee powder processing, respectively. AP contains high pectin and polyphenols and possesses important techno-functional properties such as rheological and water holding capacities (Calvete-Torre et al., 2021). Similarly, CSS is a good source of protein and polyphenols. Both AP and CSS possess high dietary fibre contents, as outlined previously, consisting of 78-89% and 86% dietary fibre, respectively. This could make them strong potential functional ingredients for employment in meat products. Previous chapter work using these functional ingredients (See Chapter 3, Section 3.1-3.6, page 68-81) showed that they could be used to improve the techno-functional properties when incorporated in Irish breakfast sausages as added phosphate replacers. However, as phosphate could not be replaced in our previous chapter, there was a requirement to implement additional and new strategies to improve the techno-functional properties of these two fibre-rich ingredients. From an extensive scientific literature review, no studies have reported the US improvement of techno-functional properties of AP and CSS to date. Thus, this study focuses on treating two novel fibre-rich ingredients (AP powder and CSS powder) with different US treatments and subsequently analysing the post-treatment characterisation changes in their physical and chemical properties, specifically those techno-functional properties interest for meat-based product applications.

4.2 Methods and materials

4.2.1 Sample preparation

Industrial co-products AP was sourced from Muns Agroindustrial S. L. (Lleida, Spain), while CSS was sourced from Illycaffé S.p.A (Trieste, Italy). Ingredient samples were

oven-dried at mild temperature (to avoid the degradation of bioactive compounds) to 40 ^{o}C until constant weight and finely powdered using the laboratory mill (Perten Labmill 3100, PerkinElmer, Waltham, Massachusetts USA). The powdered samples are stored in sealed pouches at 4 °C.

4.2.2 Sample treatment

Suspensions containing a 10% (w/v) (based on previous works, where higher concentrations were too viscous) of finely powdered AP and CSS were made separately using distilled Milli Q water. The solutions were well stirred using the magnetic stirrer (Hei-Mix S, Heidolph Instruments GmbH & Co.KG, Schwabach, Germany). They were stored at 4^{o}C for one hour as pre-treatment sample preparation to aid the complete hydration and solubilisation of the ingredients. The solutions were then treated with a low-frequency and high power (20 kHz, 250 W) US treatment (UIP1000hdT, Hielscher Ultrasound technology, Germany) for 15 and 30 mins. The selected time intervals were based on the study by Köhn et al. (2016), where the meat-based ingredients were treated with the US for 15 and 30 minutes. The US treatment was conducted in a temperature-controlled environment, where the temperature of the solutions was maintained below 20^{o}C during the entire treatment period using jacketed glass beakers attached to a recirculating chiller (Lauda Brinkmann Ecoline RE104, Delran, New Jersey, USA). The US-treated AP and CSS solutions were freeze-dried (-25^{o}C/30^{o}C, pressure ~ 0 bar) in an FD 80 model freeze dryer (Cuddon freeze-dry, New Zealand). The powders were stored in air-tight containers at 4^{o}C for further characterisation analysis. US-treated and freeze-dried AP and CSS powders were analysed for changes in water and oil absorption capacity, colour changes, particle size modification, viscosity changes, and proximate control changes against the untreated control powder samples. Although the freeze-dried products retain most of their properties, improperly selected parameters could cause deterioration or modifications to the techno-functional properties. This can be controlled by choosing the parameters that do not melt the water since the liquid water could cause rheological changes (Nowak & Jacubcyzk, 2020). Henceforth, in this study, to eliminate the effect of freeze-drying on potential characterisation changes, both AP and CSS powders were firstly suspended in Milli Q water, then

freeze-dried without US treatment, and then compared with the treated samples and control sample. In total, two controls (AP control and CSS control), two freeze-dried samples (APFD and CSFD), two samples US-treated for 15 mins (AP 15 mins and CSS 15 mins), and two samples the US-treated for 30 mins (AP 30 mins and CSS 30 mins) were analysed. All treatments were repeated separately on three occasions for statistical validation.

4.2.3 Sample Analysis

4.2.3.1 Proximate compositional analysis

The total proximate composition for both powders was determined using the official AOAC methods. Moisture content in the sample powders was determined according to AOAC official method 925.10 (AOAC, 1925) using the TGM800 Thermogravimetric Moisture determinator (LECO, USA). Fat percentage in the powders was measured using the Oracle NMR Smart Trac rapid Fat analyser (CEM Corporation, USA) using AOAC official methods 985.15 (AOAC, 1991). The total protein content in the powders was determined using a LECO FP628 (LECO Corp, MI, USA) nitrogen analyser based on the Dumas method, and according to AOAC method 992.15 (AOAC, 1992), a conversion factor of 6.25 was employed for calculation. Inorganic ash content was determined using a Gallenkamp Muffle Furnace, AOAC Official Method 936.07(AOAC, 1936), pre-drying overnight at 550°C (Gallenkamp, UK).

4.2.3.2 Total dietary fibre content

The total dietary fibre (TDF), including insoluble dietary fibre (IDF) and soluble dietary fibre (SDF), was determined according to AOAC 991.43 (AOAC, 1994) (enzymatic–gravimetric method) using Ankom automated dietary fibre analyser (Ankom, USA). Samples were dispersed in MES-TRIS buffer and were treated sequentially with a heat-stable α-amylase for 30 min at 100°C while being treated in sequence with a protease for 30 min at 60°C, and finally treated with an amyloglucosidase for 30 min at 60°C. Finally, the mixture was filtered in pre-weighed crucibles, and the IDF was measured directly. The SDF was precipitated with 95% ethanol at 4 × volume from the filtrated portion and then filtered. Both residues (insoluble and soluble) were washed with 78% ethanol, 95% ethanol, and acetone, respectively, and then dried, weighed, and corrected by protein and ash content. Protein content was

determined with the Dumas method using a LECO FP-628 (LECO Corp., MI, USA) protein/nitrogen analyser, and 6.25 as the conversion factor for N (AOAC method 992.15 (AOAC, 1992)). Ash was determined by AOAC method 936.07 (AOAC, 1936), where samples were placed in a Gallenkamp muffle furnace (Gallenkamp, UK) at 550°C overnight.

4.2.3.3 Water absorption and Oil absorption capacity

The water absorption capacity (WAC) and oil absorption capacity (OAC) of the treated ingredients were evaluated based on the method by Garcia-Vaquero, Lopez-Alonso, and Hayes (2017), with some modifications. A sample of 0.5g of each ingredient was dispersed in 10g of water or vegetable oil using a vortex mixer and then allowed to stand for 30 min. The tubes were then centrifuged at 2200 X g for 30 min in a Sorvall Lynx 6000 centrifuge (Fischer Scientific Ireland, Dublin, Ireland). The supernatant was decanted, and the tubes were then allowed to drain. After drying, the tubes were weighed again with water/oil-absorbed ingredients. The water/oil absorption capacity of the ingredients was then calculated using the formula,

$$\text{Water/ Oil absorption capacity} = \frac{W_2 - W_1}{W_0} X\ 100 \quad (1)$$

Where W_0 is the weight of the dry sample (g), W_1 is the weight of the tube with the dry sample in it (g), and W_2 is the weight of the tube with centrifuged sediment (g). Readings were measured in triplicates.

4.2.3.4 Colour Evaluation

The effect of US treatment on the colour characteristics of different ingredients was measured using a Chroma meter CR410 (Konica Minolta, Tokyo, Japan)**.** Readings were measured in CIE L* (lightness), a* (redness/greenness), and b* (yellowness/blueness) system.

The colour of the freeze-dried AP and CSS powders was compared with the colour of control powder samples. Colour comparisons were performed using the ΔE^*_{ab} values, which indicate the distance between any two colours in CIELAB space defined by its three orthogonal coordinates L^*, a^* and b^*. The total colour difference ΔE^*_{ab} values were calculated using the formula equation associated with the measurement

(Salgado, Fernández, Drago, & Mauri, 2011) and differences would only be apparent to experienced observers if 0<ΔE<2 and clear differences in colour for inexperienced observers could be observed if ΔE>2.5. The equation is as follows:

$$\Delta E^*_{ab} = \sqrt{(L^*_C - L^*_1)^2 + (a^*_C - a^*_1)^2 + (b^*_C - b^*_1)^2} \quad (2)$$

L^*_C, a^*_C and b^*_C were the colour attributes of the control sample and L^*_1, a^*_1, and b^*_1 were the colour attributes of the treated samples to compare.

4.2.3.5 Particle-size distribution

The particle-size distribution of both ingredients was studied using laser diffraction employing a particle-size analyser (Malvern Mastersizer 3000, Malvern instruments Limited, UK) equipped with a Hydro 2000SM sample dispersion unit (Malvern Instruments Limited, UK) at 25°C. Particle size was expressed with the surface mean diameter (D [3, 2]) and the volume mean diameter (D [4, 3]). A refractive index of 1.52 and an absorption index of 0.01 were used.

4.2.3.6 Rheology

A 6% (w/v) of the ingredient dispersions were made using distilled water and stirred with a magnetic stirrer (Thermo Fisher Scientific). The rheological behaviour of the ingredients was studied using an oscillatory rheometer Anton-Paar (Anton–Paar Physica MCR301, Anton-Paar, Austria) coupled to a water bath (Julabo AWC 100, Germany) at 20°C using the measuring system PP50 (parallel plates; plates diameter = 49.965mm) with a 0.5mm gap. Equilibration was initiated for 5 min, followed by the pre-shear treatment at 1 s^{-1} for 10 s. It was followed by the continuous shear rate ramps from 0.1 to 400 s^{-1} over 2 min (Gabiatti et al., 2020). The shear stress of the samples was measured throughout the experiment. The Power law (Eq. (3)) model was fitted to the experimental data using the rising curve:

$$\sigma = K \cdot \gamma^n \quad (3)$$

where σ is the shear stress (Pa), γ is the shear rate (s^{-1}), K is the consistency index (Pa. s^n), and n is the flow behaviour index (dimensionless).

4.2.4 Statistical analysis

Results from the different treatments were compared by one-way ANOVA followed by Post-Hoc analysis using Tukey's difference of $P < 0.05$ employing IBM Statistical Package for the Social Sciences (SPSS, v.24) (IBM Corp., USA). The power-law model for viscosity co-efficient was fitted using the Sigmaplot version 14.0 (Systat Software, San Jose, CA). All experiments were repeated three times, and results were expressed as mean ± standard deviation.

4.3 Results and Discussions

4.3.1 Proximate composition and total dietary fibre content

The proximate content results and the total dietary fibre content for both ingredients are summarised in Table 4-1. It was observed that US treatment did not produce any significant difference in the ash content of both the ingredients (AP and CSS), irrespective of the treatment duration. The total ash content of the CSS powder (4.78- 5.11%) was higher when compared to that of AP (1.37–1.47%). This could be due to a larger composition of inorganic minerals, including; potassium, calcium, magnesium, sulphur, phosphorus, iron and others. These minerals present in CSS are mostly essential micronutrients responsible for various metabolic and physiological functions in the human body (Ballesteros, Teixeira, & Mussatto, 2014). Similar to ash content, the US did not produce any significant trend or difference in the fat content of both ingredients. It was observed that both fat content of AP (0.48–0.77%) and CSS (1.03-1.35%) ingredients remained almost the same irrespective of the US and freeze-drying treatment. US application decreased ($P<0.05$) moisture content in both food ingredients studied. The moisture content of both AP (7.88%) and CSS (8.65%) significantly decreased when treated with US for 15 mins (AP –6.0 %; CSS–7.5%) and 30 mins (AP 6.5%; CSS–7.3%). This could be probably explained by the drying and cooking effect caused by the cavitation mechanism of US treatment, which can remove moisture (García-Pérez, Cárcel, Benedito, & Mulet, 2007; Mulet, Cárcel, Sanjuán, & Bon, 2003). It was observed that the moisture content of AP freeze-dried samples was similar to that of US-treated samples, whereas there was a significant difference ($P<0.01$) between CSS freeze-dried samples and US-treated samples. This

observation for CSS can be explained by the drying kinetics facilitated by US treatment (Zhu, Zhang, Hinds, Sun, & Tiwari, 2021).

According to studies like those carried out by Ozuna, Paniagua-Martínez, Castaño-Tostado, Ozimek, and Amaya-Llano (2015), and Téllez-Morales et al. (2020), US has been used to improve the techno-functional properties of many proteins. Protein content results for both AP and CSS showed increased protein content when both were treated with the US. Increasing protein content in AP was observed when treated with the US. However, the increase was insignificant. There was a clearer CSS case, and the US application increased ($P<0.01$) protein content. This increase in protein content of the samples can be attributed to the reduced moisture content produced because of US drying kinetics (Zhu et al., 2021). It was evident from the results of CSS samples treated with the US for 15 and 30 mins, where the moisture content reduction had resulted in protein content concentration. On analysing the post-hoc studies, it was observed that there was no significant variation in protein content between samples where the US had been applied for different treatment durations. Thus, US treatment increased ingredient protein content but only by physically lowering the moisture content of ingredients studied.

Regarding the dietary fibre content of the ingredients investigated, US treatment had no significant effects. The total dietary fibre (TDF) involving insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) did not vary significantly with US treatment in the case of AP ingredients. The IDF ranged from 39.0-42.0%, while the SDF ranged from 11.0 –12.0%. Differences in fibre content can be obtained by varying the milling time. A reduction in particle size could result in IDF conversion to SDF due to modifications in the structure and physicochemical properties (Lu et al., 2020). This redistribution of IDF to soluble fractions was explained by the destruction of cellulose, hemicellulose, and exposure of hydrophilic groups produced by milling (Lu et al., 2018). In the case of CSS, US treatment significantly affected SDF ($P<0.05$). However, no significant effect was observed for TDF content ($P>0.05$), concluding that US treatment did not alter the studied ingredients' fibre content. Fibre is the main component of both ingredients under study, and this high fibre content is essential in numerous respects. The techno-functional properties of these rich

dietary fibres in both ingredients make them potential new food ingredients for the food and beverage industries (Iriondo-DeHond et al., 2019). For example, the techno-functional properties of dietary fibres in these ingredients can be used to improve emulsion stability, sensory properties, and rheological properties of meat products (Das et al., 2020). The high SDF content in CSS can produce probiotic foods by promoting bacterial growth (Borrelli, Esposito, Napolitano, Ritieni, & Fogliano, 2004).

4.3.2 Water and Oil absorption Capacity

The water absorption capacity (WAC) and oil absorption capacity (OAC) are ingredient properties that must be understood if those ingredients are to be used in a processed meat product and can be defined as the ability of the ingredient to absorb or withhold water or oil, respectively, even after the application of an external force such as temperature (Luo et al., 2017). The WAC values are responsible for the hydration properties of food ingredients, whereas the OAC determines the ability of an ingredient to bind with fat during food processing. Ingredients with excellent oil and water binding properties can be used in food formulations such as meat and oil emulsion products (Gabiatti et al., 2020; Navarro-González, García-Valverde, García-Alonso, & Periago, 2011). It is reported that high TDF content in ingredients is responsible for the increased WAC of the same ingredients (Raghavendra, Rastogi, Raghavarao, & Tharanathan, 2004). Both WAC and OAC values for ingredients are dependent upon the particle size of these ingredients. It is reported that the smaller the particle size, the higher the chances of increased WAC since the smaller particles possess a higher packing density. Additionally, the amount of OAC is dependent on ingredient properties like surface properties, overall charge density, and the hydrophobic nature of the ingredient itself (Ballesteros et al., 2014; Murthy & Naidu, 2012). Téllez-Morales et al. (2020) outlined the positive impacts of US application on the WAC and OAC of food ingredients.

Table 4-1: The effect of Ultrasound on the proximate composition and total fibre content of the ingredients

	Apple Pomace					Coffee Silverskin				
	AP Control	**AP Freeze-dried**	**AP US 15 min**	**AP US 30 min**	**p-value**	**CS Control**	**CS Freeze-dried**	**CS US 15 min**	**CS US 30 min**	**p-value**
Ash (%)	1.42 ± 0.17	1.37 ± 0.15	1.47 ± 0.06	1.38 ± 0.21	0.73	4.83 ± 0.32	4.78 ± 0.27	5.11 ± 0.24	4.91 ± 0.42	0.31
Protein (%)	4.49 ± 0.35	4.43 ± 0.19	5.03 ± 0.52	5.00 ± 0.68	0.19	15.68 ± 0.39^{b}	16.01 ± 0.36^{b}	17.45 ± 0.69^{a}	17.10 ± 0.61^{a}	**< 0.01**
Moisture (%)	7.88 ± 1.51^{a}	6.05 ± 0.94^{b}	6.00 ± 0.71^{b}	6.50 ± 1.01^{a,b}	**0.02**	8.65 ± 0.26^{a}	8.13 ± 0.33^{a,b}	7.53 ± 0.80^{a,b}	7.29 ± 1.37^{b}	**0.04**
Fat (%)	0.48 ± 0.34	0.62 ± 0.27	0.67 ± 0.46	0.77 ± 0.27	0.54	1.03 ± 0.60	1.30 ± 0.37	1.35 ± 0.45	1.09 ± 0.51	0.61
TDF (%)	51.60 ± 1.57	53.01 ± 4.17	51.44 ± 3.00	51.58 ± 3.82	0.77	69.89 ± 2.63	66.43 ± 2.12	69.62 ± 3.45	77.94 ± 12.69	0.12
IDF (%)	40.46 ± 0.40	41.26 ± 3.18	40.34 ± 1.76	39.60 ± 4.15	0.84	62.90 ± 2.15	58.90 ± 1.62	61.92 ± 4.61	69.62 ± 14.13	0.59
SDF (%)	11.14 ± 1.69	11.74 ± 1.51	11.05 ± 3.12	11.99 ± 1.75	0.82	6.95 ± 1.31^{a,b}	7.53 ± 1.32^{b}	7.70 ± 2.29^{a,b}	8.32 ± 1.69^{a}	**0.04**

AP – Apple pomace; CS – Coffee silver skin; US – Power ultrasound treatment; TDF – Total Dietary fibre; IDF – Insoluble dietary fibre; SDF – soluble dietary fibre

a – c Mean values without the common letter in each testing parameter are significantly different (P<0.05).

Application of US to food ingredients physically alters their matrices so that there is an opening of structures, an increase in electrical charge, and an exposure in hydrophilic and hydrophobic groupings, thereby changing and improving functionalities like WAC and OAC; much like that achieved through the addition of salt to processed meats. The US impact on WAC and OAC for experimental ingredients used in this study is shown in Table 4-2. US application had a more significant overall effect on OAC than WAC. It was observed that the WAC and OAC of AP powder significantly ($P<0.01$) increased owing to the application of US. It can be seen from the results that both WAC and OAC for AP also increased with increasing treatment duration. The WAC (3.50g water/g dry sample) and OAC (1.64g oil/g dry sample) values for AP treated with the US for 30 min were significantly higher ($P<0.01$) than the one treated for 15 mins (WAC– 3.13g water/g dry sample; OAC– 1.36g oil/g dry sample). Thus, US application significantly impacted WAC and OAC for the AP ingredient (Table 4-2).

Table 4-2: Effect of ultrasound treatment on the water and oil absorption capacity of the ingredients

	Apple Pomace				
	AP Control	**AP Freeze-dried**	**AP US 15 min**	**AP US 30 min**	**p-value**
WAC (g water/g dry sample)	2.92 ± 0.15^{c}	$2.95 \pm 0.09^{b,c}$	3.13 ± 0.20^{b}	3.50 ± 0.19^{a}	< 0.01
OAC (g oil/ g dry sample)	1.03 ± 0.04^{c}	1.22 ± 0.14^{b}	1.36 ± 0.11^{b}	1.64 ± 0.16^{a}	< 0.01
	Coffee Silverskin				
	CS Control	**CS Freeze-dried**	**CS US 15 min**	**CS US 30 min**	**p-value**
WAC (g water/g dry sample)	3.19 ± 0.30	3.03 ± 0. 24	3.49 ± 0.23	3.62 ± 0.22	0.07
OAC (g oil/ g dry sample)	2.84 ± 0.19	2.86 ± 0.46	3.34 ± 0.73	3.58 ± 0.41	0.25

Where WAC – water absorption capacity; OAC – Oil absorption capacity; AP – apple pomace; CS- coffee silver skin; US – power ultrasound treatment

a– c Mean values without the common letter in each testing parameter are significantly different ($P<0.05$).

Both the WAC (3.03–3.62g water/g dry matter) and OAC (2.84–3.58g oil/g dry matter) were higher in CSS when compared with the WAC (2.92-3.50g water/g dry matter) and OAC (1.03–1.64g oil/g dry matter) values for AP. An increasing trend was observed in both WAC and OAC values for CSS when treated with the US for 15 and 30 mins (Table 4-2). However, the trends observed were not significant to prove the effect of US on the ingredient's WAC and OAC properties. Since the WAC and OAC depend on the particle size (Ballesteros et al., 2014), this non-significant increase in WAC and OAC can be attributed to the close range of particle size of the US-treated CSS ingredients with that of control. In general, it was observed from the data that US treatment had positive impacts on the WAC and OAC of ingredients, which plays a vital role in the nutritional and sensory aspects of food products, mainly processed meat products (Tiwari and Cummins, 2011).

4.3.3 Particle-Size Distribution

The particle size distribution results were presented in Table 4-3 in terms of surface mean diameter D (3, 2) and volume mean diameter D (4, 3). It was understood that the most significant ingredient particles had the greatest influence on volume mean diameter D (4, 3). In contrast, the smaller particles impacted the surface mean diameter D (3, 2). It was found that D (4, 3) values for AP had a significant ($P<0.01$) increasing effect due to the US treatment. The D (4, 3) values for AP treated with the US for the 15 mins (150.67 µm) were higher than AP treated with the US for 30 mins (138.33 µm). An opposite trend was observed in CSS, where the D (4, 3) values for CSS treated with the US for 15 mins (169.67 µm) and 30 mins (193.33 µm) decreased when compared with the control (201.67 µm). The possible explanation for this can be attributed to the shrinkage effect produced in the CSS due to freeze-drying (Nowak & Jacubcyzk, 2020).

Results showed that there were a significant increase in D (3, 2) values for both AP ($P<0.01$) and CSS ($P<0.01$) when treated with the US for 15 and 30 mins. In the case of AP, D (3, 2) values for both treatment durations were similar (70 µm). However, for CSS, although D (3, 2) values for both treatment durations were significantly different compared to control values, the range of difference was not higher than AP. This explains the non-significant increase in WAC and OAC values of CSS. Also, The

distribution of D (3, 2) values for AP (30–70µm) and CSS (96.4–118.3µm) was so small that this explains the increased values observed for WAC and OAC for both ingredients since the parameters are dependent on the particle surface area, and smaller particles trap more water/oil due to their larger surface area and more open structure (Chen et al., 2019; Lu et al., 2020). However, US treatment, which activates surface area, also explains the increased WAC and OAC values observed for ingredients compared with control samples of a smaller size. Thus, the application of the US produces changes in the particle size distribution of the ingredients. These changes can be attributed to the modifications in the rheological behaviour and technological properties of TDF and the WAC and OAC of the ingredients (Bengtsson & Tornberg, 2011).

4.3.4 Rheological Study

Food rheology is a valuable tool for many food processing applications and involves studying the deformation and flow of foods under well-defined conditions. Food rheology is often confined to liquid food materials. Still, there is an increased interest in learning the responses of both liquid and solid food materials to applied stress and strains. It is defined as studying foods' elastic and plastic properties (McKenna and Lyng, 2003). Finally, viscosity refers to the internal resistance between the molecules against the fluid flow (Liu et al., 2021). It is an essential characteristic of a fibre solution as it represents its behaviour pattern during various industrial processes such as mixing, grinding, coating, etc. (Gabiatti et al., 2020). The apparent viscosity at a shear rate of 100 s^{-1} was used to calculate the significance, as it would represent the process behaviour as pumping, shaking, and chewing (McClemens et al. 2016).

Table 4-3: Results of particle size distribution analysis and colour analysis of apple pomace and coffee silverskin powders

	Apple pomace				Coffee silver skin			
	AP Control	**AP US 15 min**	**AP US 30 min**	**p-value**	**CS Control**	**CS US 15 min**	**CS US 30 min**	**p-value**
D (3,2) µm	30 ± 0.00^{b}	70 ± 0.00^{a}	70 ± 0.00^{a}	**< 0.01**	96.36 ± 9.80^{b}	109.67 ± 3.05^{a,b}	118.33 ± 1.15^{a}	**< 0.01**
D (4,3) µm	82.93 ± 2.65^{c}	150.67 ± 1.52^{a}	138.33 ± 4.72^{b}	**< 0.01**	201.67 ± 37.87	169.67 ± 8.62	193.33 ± 1.52	0.223
D 90 µm	193.33 ± 8.08^{c}	290.00 ± 1.73^{a}	266.67 ± 8.38^{b}	**< 0.01**	335.67 ±34.64^{a,b}	292.00 ± 21.07^{b}	346.33 ± 4.72^{a}	**0.037**
L*	30.88 ± 3.60^{a}	24.75 ± 1.16^{b}	25.56 ± 3.34^{b}	**< 0.01**	20.46 ± 0.85^{b}	24.04 ± 2.38^{a}	23.06 ± 1.51^{b}	**< 0.01**
a*	5.85 ± 0.62^{b}	6.84 ± 0.34^{a}	6.75 ± 0.49^{a}	**< 0.01**	6.50 ± 0.24^{a}	6.39 ± 0.20^{a,b}	6.16 ± 0.15^{b}	**0.007**
b*	21.35 ± 0.25^{b}	23.05 ± 0.95^{a}	22.94 ± 1.40^{a}	**0.001**	21.98 ± 0.56	21.81 ± 0.08	21.52 ± 0.80	0.308
ΔE		6.43	5.42			3.58	2.66	

$^{a-c}$ Mean values without the common letter in each testing parameter are significantly different (P<0.05).

Table 4-4: Consistency coefficient (K), flow behaviour index (n) and apparent viscosity of apple pomace and coffee silverskin ingredients treated with Ultrasound for 15 and 30 mins

	AP Control	**AP US 15 min**	**AP US 30 min**	**CS Control**	**CS US 15 min**	**CS US 30 min**
Consistency co-efficient K (Pa s ^{n})	2.36 X 10^{-2}	1.26 X 10^{-2}	1.09 X 10^{-2}	2.76 X 10^{-1}	2.24 X 10^{-2}	1.49 X 10^{-2}
Flow behaviour index n	0.623	0.744	0.856	0.194	0.674	0.734
R- squared R^2	0.897	0.950	0.982	0.726	0.964	0.965
Apparent viscosity at a shear rate of 100 s^{-1} (Pa. s)	3.94 ± 2.52	3.26 ± 0.53	4.33 ± 1.14	3.41 ± 1.93	2.94 ± 1.51	3.87 ± 1.47

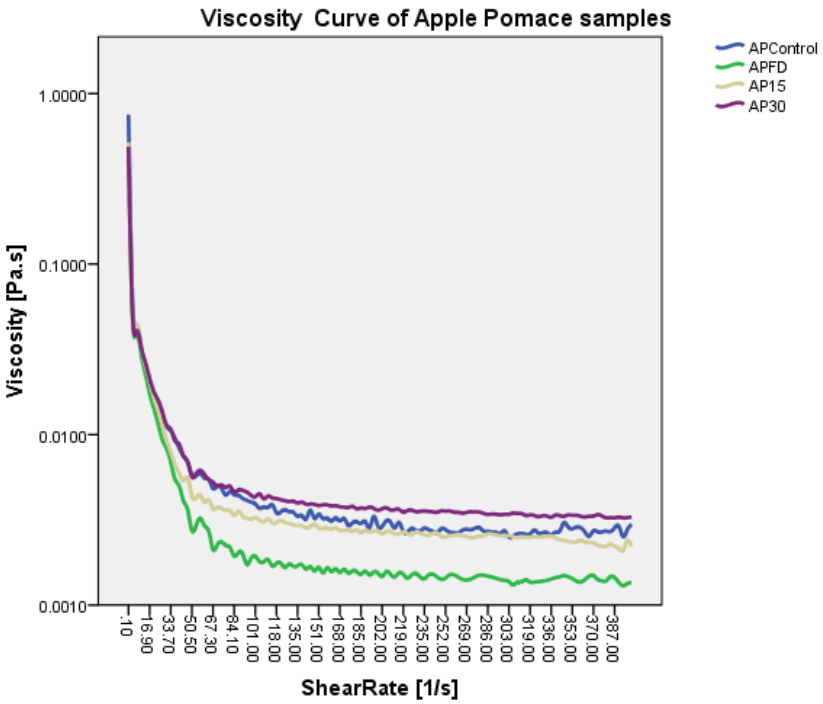


Figure 4-1: Viscosity curve of the apple pomace ingredient obtained from high-speed homogenisation using Anton Paar rheometer.

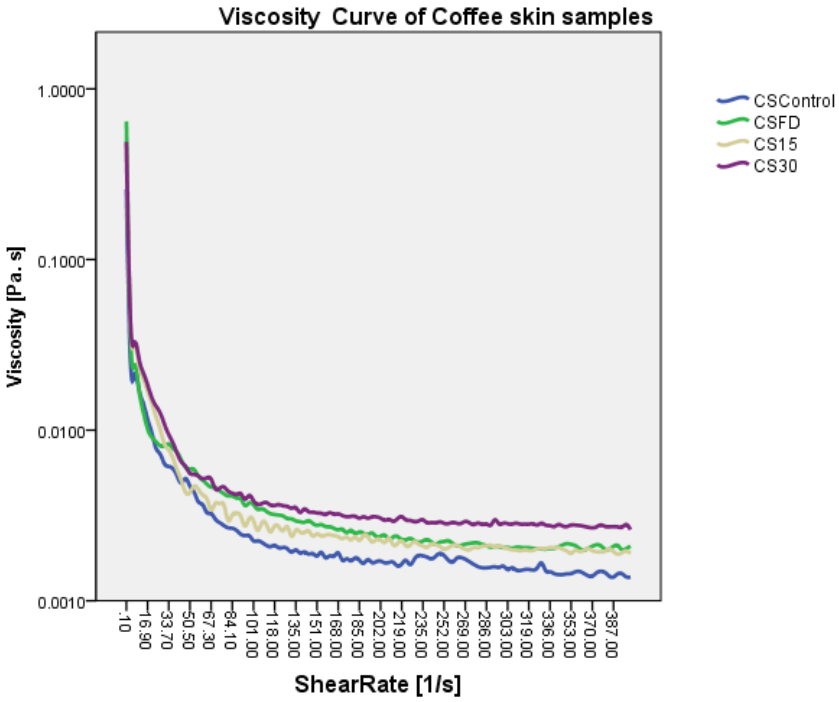


Figure 4-2: Viscosity curve of the coffee silverskin ingredient obtained from high-speed homogenisation using Anton Paar rheometer.

In this study, Figures 4-1 and 4-2 show the ingredients' viscosity curve against AP and CSS shear stress, respectively. For both ingredients investigated in this study (from Figures 4-1, 4-2), it was observed that viscosity decreased with an increased shear rate. In the study of Gabiatti et al. (2020), the fibre solutions were thixotropic, i.e. the longer the ingredient underwent stress, the lower the viscosity until it reached an equilibrium level. It was observed from the viscosity curve that the apparent viscosity of the samples increased when they were treated with the US for 30 mins for both AP and CSS samples. There was a definite increase in the apparent viscosity at 100 s^{-1} shear rate; however, the increase was not significant enough to prove the effect of US treatment for both ingredient samples. The power-law model fitted data from Table 4-4 showed that both samples possessed shear thinning properties ($n<1.0$). Analysis showed that the model resulted in excellent fits with high correlation coefficients (R^2), which increased with US treatment duration (R-square of 0.95 and 0.98 for AP treated with the US for 15 and 30 mins, respectively; 0.96 for CSS treated with the US for 15 and 30 mins, respectively). The consistency coefficient (K), which represents the viscous nature of the ingredients (Simuang, Chiewchan, & Tansakul, 2004), decreased with US treatment for both samples, along with the increase in flow behaviour index (n). It was referred from the study by Wang, Sun, Li, Adhikari, and Li (2018) that the viscosity of fibre solutions increased with an increase in product fibre content. Knowing the viscoelasticity of the ingredients is of high importance as they can be used to design and predict product stability (Augusto, Ibarz, & Cristianini, 2013).

4.3.5 Colour Analysis

Colour parameters for food ingredients are critical since they primarily affect the colour of the finished food products. Additionally, the product's colour is responsible for consumers' perception and reception of the food products (Banaś, Korus, & Korus, 2018). The Colour analysis of the ingredient samples is listed in Table 3. Research showed that for CSS, US treatment significantly increased ($P<0.01$) lightness values (L*) in samples, while redness values (a*) for samples decreased significantly after treatment. There was no significant change in yellowness (b*) values. The difference in the colour can be attributed to the freeze-drying and the cooking effect

produced by the US that increases the darkness of the samples. However, in this study, the results contrasted with Vimercati et al. (2022) reports, where the freeze-drying of the CSS increased the darkness values.

For AP, an opposite trend to that observed for CSS was determined. There were significant differences between control and US-treated AP samples in terms of L*, a* and b* values. It was found that L*(lightness) values for AP samples significantly decreased ($P<0.01$) with US treatment for 15 and 30 mins. On analysis, it was determined that both yellowness (b*) and redness (a*) values for samples increased significantly ($P<0.001$) with US treatment. This increase in the darkness of the sample can be explained by the release of carotenoids by US treatment (apple peel consists of carotenoid and chlorophyll pigments) (Hussain, Sharma & Bhat, 2021) and the browning effect caused by freeze-drying (Nowak & Jacubcyzk, 2020).

Further sample analysis of colour difference (ΔE) values showed a clear difference in observed colour values. When the colour difference (ΔE) values are more than 2, then a clear difference in colour between samples can be followed by an ordinary observer (Mokrzycki & Tatol (2011). It was observed from Table 4-3 that AP samples treated with the US for 15 (6.43) and 30 mins (5.42); CSS samples treated with the US for 15 (3.58) and 30 mins (2.66), both had colour difference values greater than 2.5 which signifies that the application of US treatment produced colour changes in the food ingredients when compared with control samples. Thus, the application of the US had a varied effect on both ingredient samples. These effects can be used to produce a product of the desired colour in a controlled treatment procedure.

4.4 Conclusion

US treatment of food ingredients has attracted scientific and commercial interest owing to its ability to improve various techno-functional properties associated with these ingredients. Understanding the optimal conditions required for the US to influence and fully exploit desirable ingredient functionalities is essential. The research undertaken in this study demonstrated the physicochemical changes induced in fibre-rich AP and CSS powders when treated with US (20kHz, 250W) being more evident at longer treatment times (15 vs 30 minutes). The application of US on both these ingredients produced enhanced modifications in their structural and

physicochemical properties, such as improvements in; WAC, OAC and viscosity values, which can be attributed to alterations in ingredient particle sizes as impacted by the US; although the conditions employed in this research produced better and more significant improvements for the functional properties determined for AP as an active ingredient. This study suggests that further work should be undertaken to assess the impact that US application has on enhancing ingredient functionalities and in the revalorisation of food co-products as functional ingredients in food formulations, specifically in meat products such as sausages and burgers.

Chapter 5. A comparative study on the effect of ultrasound-treated apple pomace and coffee silverskin powders as phosphate replacers in Irish breakfast sausage formulations

Overview

This chapter evaluates the ability of US-treated AP and CSS to improve the quality of three phosphate-reduced Irish breakfast sausage formulations obtained from chapter 3. Physicochemical properties of sausage formulations such as water holding capacity (WHC), cook loss, proximate content, lipid oxidation, low-field nuclear magnetic resonance (LF-NMR), colour and textural parameters were used to analyse the effect of US-treated AP and CSS and a comparison methodology used to evaluate the effects. A manuscript from this chapter has been sent to the journal for publication.

- Thangavelu, K. P., Tiwari, B. K., Kerry, J. P., & Álvarez, C. A comparative study on the effect of ultrasound treated apple pomace and coffee silver skin powders as phosphate replacers in Irish breakfast sausage formulations. *Foods, 11* (18), 2763.

5.1 Introduction

Meat and meat products are widely consumed and play an essential role in consumer food choice since they provide nutrients like high biological value proteins, minerals (zinc, selenium, iron and phosphorus), vitamins (B_{12} and other B complex vitamins), essential amino acids and fatty acids (Illippangama, Jayasena, Jo, & Mudannayake, 2022). However, in particular, consumption of processed meat products has been questioned over the past decade due to numerous reasons, in particular, has been due to health concerns linked to the composition of processed meat products owing to high salt contents, fat levels and make-up, synthetic processing additives etc. In addition, the formation of several undesirable components has suggested to have serious health effects in humans including the cancer formation (Handel et al., 2021). The current and overuse of such processing ingredients have been linked to several profound health implications, such as; cardiovascular problems, obesity, kidney-related problems and certain cancers (Domingo & Nadal, 2017; Pérez-Montes, Rangel-Vargas, Lorenzo, Romero, & Santos, 2021). In recent years, consumer demands for healthier, clean-label meat products containing no synthetic additives have forced processed meat industries to reformulate their products to address consumer concerns.

Phosphates comprise one such synthetic additive grouping and are added to processed meat products as; emulsifiers, stabilisers, sequestrants, and thickeners and provide various functionalities, such as; increased pH, decreased cook loss, improved water holding capacity (WHC) and enhanced textural and sensory properties (See Chapter 1, Section 4, page 8). Alkaline phosphates work synergistically with sodium chloride (NaCl) in extracting myofibrillar proteins and improve meat products' oxidative and microbiological stability (Pinton et al., 2021) as they function as metal chelators and reduce water activity, respectively. Excess consumption of phosphates, higher than the acceptable daily intake (ADI) of 40 mg/kg for a healthy adult (Younes et al., 2019), is likely to cause fatal kidney-related problems in people with chronic kidney disorders (CKD) (Winger, Uribarri, & Lloyd, 2012). Phosphate addition reduces calcium absorption, even in healthy individuals, weakening bone strength (Takeda, Yamamoto, Yamanaka-Okumura, & Taketani,

2014). This has resulted in incorporating alternative and natural agro-industrial by-products and/or techno-functional food ingredients into meat and meat products as a replacement for phosphate additives, thereby reducing the possible harmful effects on consumers and facilitating clean labelling practices.

Dietary fibres are a type of techno-functional ingredients that can be added to meat products due to their various health benefits, like improving gastrointestinal function, reducing the incidence of breast and colon cancer, lowering cholesterol absorption, preventing cardiac diseases, and decreasing the risks of obesity (Hathwar et al., 2012; Ren, Feng, Zhang, & Wang, 2021). Additionally, dietary fibres in meat products improve WHC, emulsion stability, cook loss, and textural and rheological characteristics (Illippangama et al., 2022; Talukder, 2015). Several studies reported this intrinsic techno-functional properties of several dietary fibres in meat products (Câmara et al., 2020; El Zeny, Essa, Bisar, & Metwalli, 2019; Han & Bertram, 2017).

Apple pomace (AP) and coffee silverskin (CSS) are the two dietary fibre-rich agro-industrial by-products discussed in this study as phosphate replacers in Irish breakfast sausages. AP, obtained as a by-product from apple juice industries, consists of pectin, polyphenols, vitamins, and organic acids. AP has a rich content of total dietary fibre (TDF) of 60-90% on dry basis, primarily insoluble, contributing to better rheological and WHC properties (Figuerola et al., 2005). CSS is obtained as a by-product of the coffee roasting process, and it is rich in proteins, minerals, and TDF on dry basis (86%), which are also mainly insoluble. Both AP and CSS are reported to possess excellent antioxidant properties (Belmiro, Oliveira, Tribst, & Cristianini, 2022). The rich source of dietary fibres in both AP and CSS makes them potential phosphate replacers in meat products. Previous chapter study (See Chapter 3, Section 4, page 8), using AP and CSS mixture in Irish breakfast sausages, showed that phosphates could be reduced to significantly lower quantities (~ up to 80%) without significantly affecting the physicochemical and technological properties of these traditional processed meat products. However, the complete replacement of phosphates negatively affected the quality of sausages. Therefore, it was hypothesised that applying a novel approach to modify the ingredients to improve

their techno-functional properties could be used to further reduce or eliminate phosphates from sausage formulation.

Power Ultrasound (US) technology represents a non-thermal and green-processing technology that uses high-frequency sound waves to modify food ingredients physically and, thus, modifying their techno-functional properties such as water absorption capacity (WAC), oil absorption capacity (OAC), solubility, emulsification capacity, gelling capacity, swelling capacity, and foaming capacity (Téllez-Morales, Hernández-Santo, & Rodríguez-Miranda, 2020; Arzeni et al., 2012). The US works on the mechanism of cavitation that is responsible for enhancing various food-processing techniques, such as; extraction, freezing, drying, emulsification, and inactivating pathogens (Téllez-Morales et al., 2020). Previous chapter study (See Chapter 4, Section 4, page 107) when individual AP and CSS (10% w/v) suspensions were treated with US (20 kHz, 250 W) for 15 and 30 mins, it was observed that there was an improvement in the physicochemical properties such as WAC, OAC, and viscosity values. This novel modification of ingredients can be used to address the aforementioned negative sausage quality.

It was noted that very few studies had addressed the effects produced by the application of US-treated ingredients in food products. This is the first report on US-treated AP and CSS's impact on phosphate-reduced meat products. Thus, the main aim of the study is to (i) introduce the US-treated AP and CSS into phosphate-reduced Irish breakfast sausage formulations and (ii) to assess if the US-treated ingredients had improved the technological properties of the phosphate-reduced sausage formulations by comparing them with that of the sausage formulations with non-US-treated ingredients.

5.2 Methods and Materials

5.2.1 Ingredient preparation

Food co-products AP and CSS were responsibly sourced from Muns Agroindustrial S. LO. (Lleida, Spain) and Illy S. P. A. (Trieste, Italy). They were oven-dried at 40^{o}C to constant weight before being ground into finely powdered ingredients using a laboratory mill (Perten Labmill 3100, PerkinElmer, Waltham, Massachusetts, USA). Samples were stored at 4^{o}C in sealable low-density polyethylene bags until further

treatment. Both ingredients' water suspensions (10% w/v) were prepared and held at 4°C (60 mins) for complete hydration as pre-treatment preparation. All solutions were treated using high power (250 W, 20 kHz) US probe (UIP1000hdT, Hielscher Ultrasound technology, Germany) for 30 mins in a temperature-controlled setup (≤ 20°C), consisting of jacketed glass beakers attached with a recirculating chiller (Lauda Brinkmann Ecoline RE104, Delran, New Jersey, USA). Selected US parameters employed in this study were based on Chapter 4, Section 4, and page 107. US treatment of AP and CSS for 30 minutes improved the functional properties better than that treated with the US for 15 minutes. All suspensions were freeze-dried (Cuddon freeze-dry, New Zealand), and the dried powders were stored separately at 4°C in airtight plastic containers for further experimental analyses. Non-US-treated ingredients were employed with no other treatment than initial drying step.

5.2.2 Sausage production

Sausages were prepared using pork lean meat (>90%) and fat obtained from three-to-four fresh pork loins (pH 5.3 – 6.0) purchased from a local butcher shop (Gleeson Butchers, Dublin, Ireland). The lean meat and back fat was minced using a meat mincer (Meat Grinder MG510, Kenwood, UK) and was stored at 4°C throughout all stages of production. The seasoning mix (without added phosphates) was purchased from Redbrook ingredient service (Dublin, Ireland). Rusk and sodium tripolyphosphate (STPP) required for sausage manufacture were procured from All-in-All ingredients (Dublin, Ireland). Sausages, per formulation, were prepared using the mixture composition (% of w/w) of pork lean meat (58.00%), pork back fat (20.35%), water/ice (13.45%), rusk (5.75%), seasoning mix (1.45%), and different combinations (1.00%) of STPP and/or phosphate replacers (AP and CSS) for each formulation. The formulations were as follows:

- Formulation 1 - 0.20% STPP + 0.22% AP + 0.58% CSS
- Formulation 2 - 0.20% STPP + 0.00% AP + 0.80% CSS
- Formulation 3 - 0.06% STPP + 0.94% AP + 0.00% CSS

These formulations were obtained from our previous validation process, which demonstrated the phosphate-replacing ability of AP and CSS in Irish breakfast sausages (See Chapter 3, Section 3.7, page 82). The above formulations containing

STPP and non-US-treated AP and CSS were considered as control samples for this study (Since this study aimed to determine the impact of US on added recipe ingredients, traditional recipes were not included as a control since we previously demonstrated that such formulations performed better in terms of functionality). About 20 sausages (~10 cm in length; 23 mm diameter) per formulation were prepared using a meat mincer fitter with sausage filler (Meat Grinder MG510, Kenwood, UK) and the prepared sausages were chosen randomly for further analysis. The functional properties of sausage formulations containing US-treated AP and CSS were compared to sausage formulations with non-treated AP and CSS. Prepared sausages (day 0) were retail packaged using the padded black tray (h 197mm x w 155mm x d 30mm; Silverstream packaging Ltd, Cork, Ireland) heat wrapped using the cling film (gas permeability −2.5 [g 100 µm]/ [m2d]; 300mm x 300m, Prowarp, Bristol, UK), without the gas flushed. The sausage packs were then held under simulated retail chilled conditions (EXPO PT, glass door upright display cooler, Framec, Italy) at 3-5^{o}C for the storage trial and sampled at various specified time points. Sausages were analysed for WHC (day 1), cook loss (day 1), water mobility using low-field nuclear magnetic resonance (LF-NMR) (day 1), emulsion stability (day 1), texture profile analysis (TPA) (day 2), lipid oxidation (day 0, 3, 6 and 9), colour (day 2), and proximate analysis changes to examine the impact of US-treated AP and CSS in sausages. The entire study was performed in three independent trials on three different days to achieve statistical validation.

5.2.3 Proximate analysis

Homogenised sausage batters per formulation were used to analyse the proximate composition of sausages. Protein percentage in sausages was measured using the Dumas method employing LECO nitrogen content determiner (LECO FP628, LECO Corp, MI, USA) according to AOAC method 992.15, (1990) using the nitrogen to a protein conversion factor of 6.25. Moisture and fat content were determined based on AOAC 985.14 (1990) and AOAC 2008.06 (2008), respectively, using the SmartTrac5 rapid fat/moisture analyser (SmartTrac 6, CEM Corp, NC, USA). Ash was determined based on AOAC 920.153 (1920) using a 550^{o}C Gellenkamp heating furnace (Gellenkamp, UK), and sodium chloride (NaCl) content was measured from the ash

using the Bohr titration method based on AOAC 935.47 (1987). According to AOAC 991.43 (1995), total dietary fibre content was measured using the ANKOM^{TDF} Dietary fibre analyser (ANKOM Technology, NY, USA). All experiments were carried out in triplicates, and the values were averaged.

5.2.4 Measurement of WHC and cook loss of sausages

WHC of the sausages was measured using methods described by Lianji & Chen (1989), with some modifications. Raw samples of approximately 10g (weight B) were measured in a 50ml centrifuge tube and heated in a water bath for 10 mins at 90°C. Samples were removed from the tube after heating and cooled to room temperature. Cooled samples were weighed (weight C), wrapped in cheesecloth, and then placed in a 50ml centrifuge tube filled with absorbent cotton wool (filled up to 1/3 part of the centrifuge tube). Samples were centrifuged for 10 mins at 204 x g (4°C) in a Sorvall Lynx 6000 centrifuge (Fischer Scientific Ireland, Dublin, Ireland), and the test tube samples were weighed (weight A). The following equations then calculated WHC and cook loss:

$$\text{WHC}\ (\%) = 1 - \frac{(B-A)}{M}\ x\ 100 \qquad (1)$$

Where M was the total water content in sample meat calculated from the moisture values determined using the SmartTrac rapid fat/moisture analyser (SmartTrac 6, CEM corporation, NC, USA) based on AOAC method 985.14 (1990) by microwave methodology.

$$\text{Cook Loss}\ (\%) = \frac{Initial\ weight\ (B) - Cooked\ weight\ (C)}{Initial\ weight\ (B)} x\ 100 \qquad (2)$$

5.2.5 LF-NMR analysis of bound water mobility

The water mobility of raw sausage batters per formulation was measured using the LF-NMR as described by McDonnell et al. (2013) using a Maran Ultra instrument (Oxford Instruments, Abington, Oxfordshire, UK) at a resonating frequency of 23.2 MHz. Measurements were recorded in triplicate and averaged for statistical analysis.

5.2.6 Emulsion stability

The emulsion stability of three sausages per formulation was measured based on the method reported by Hughes, Mullen, and Troy (1998). The raw batter of approx. 25g

(exact weight recorded) was placed in a 50ml centrifuge tube and centrifuged at 2958 g (1 min). Samples were heated in a water bath at 70°C for 30 mins and centrifuged at 2958 g (3 mins). The supernatants were poured into pre-weighed crucibles and dried overnight at 100°C. The pelleted samples were weighed. Volumes of total expressible fluid (TEF (%)) and percentage fat were calculated as follows:

TEF = Weight of sample – weight of pellet (3)

TEF (%) = (TEF/ sample weight) x 100 (4)

Fat Exudate (%) = (Dried supernatant/ TEF) x 100 (5)

5.2.7 Texture Profile Analysis (TPA)

TPA of sausages was measured using the Instron universal testing machine model 5543 (Instron (UK) Ltd., High Wycombe, UK) attached with a compression anvil. Five sausages per formulation were cooked in a water bath for 20- 30 mins at 73.0±1.0°C until the core reached 70°C, and after that, the sausages were cooled overnight before conducting analysis. The cored (diam. 14mm x ht. 20 mm) sausages were axially compressed to 70% of their original height at a 100mm/min crosshead speed in a two-cycle compression test. Sausages were analysed for hardness (N), gumminess (N), chewiness (N), springiness (mm), and cohesion force ratio (Bourne, 1978). The values of five sausage cores per formulation were recorded.

5.2.8 Visible colour evaluation

The colour of three sausages per batch of each experimental formulation was measured using an UltraScan Pro (Hunterlab, Reston, VA, USA) dual beam xenon flash spectrometer, with a viewing port of 25.54mm and illuminant D65, 10°. Calibration was carried out using a light trap (L = 0) and a white standard tile (L =100; X = 88.69; Y = 93.58; Z = 100.45), covered in transparent PVC cling film to eliminate any colour reading effect. To maintain reading uniformity, sausages were packaged in PVC film for measuring colour. Values were expressed in L* (lightness/darkness), a* (redness/greenness) and b* (yellowness/blueness) units. The total colour difference (ΔE) between sausages was calculated using the formula of Salgado, Fernández, Drago, and Mauri (2011).

$$\Delta E^*_{ab} = \sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2} \quad (6)$$

5.2.9 Measurement of lipid oxidation

Lipid oxidation in sausages was performed using a method based on Botsoglou et al. (1994) with some modifications. Raw blended sausage (1.5g) was mixed with milliQ water (20mL) and homogenised with an Ultraturrax homogeniser (Labortechnik, Staufen, Germany) at 13500rpm (30sec). Cold trichloroacetic acid (25% TCA; 5mL) was added, followed by a gentle stirring at 4°C (15 mins). The mixture was centrifuged at 2498 g (15 mins; 4°C), and the supernatant (3.5mL) mixed with 0.6% 2-thiobarbituric acid (1.5mL) with the reaction performed in a water bath at 70°C (30 mins). TBARS was measured at 532nm using a UV-Vis spectrophotometer (Shimadzu UV -1700, Columbia, USA), and a standard curve was prepared by Maraschiello, Sárraga, and García Regueiro (1999), with results being expressed as milligrams of malondialdehyde per kilogram of sausage (mg MDA/kg sausage). Three sausage samples per formulation from days 0, 3, 6 and 9 of storage were analysed for the TBARS method.

5.2.10 Sensory Evaluation

As discussed earlier in the literature review and thesis objective sections (See Chapter 1, Section 1.7.3.1 & 1.8, page 31 & 33), the sensory evaluation of phosphate-reduced sausage formulations with US-treated AP and CSS was not carried out. This is because of the lack of standardised regulations regarding the usage of US-treated food ingredients and the safety status of their consumption.

5.2.11 Sausage scoring system

The best overall sausage formulations, with improved physicochemical properties, were determined using the scoring method used by Álvarez, Drummond, & Mullen (2018). Important parameters that are affected by the reduced phosphate concentration were considered for the scoring system, and each parameter was standardised using the equations

$$z = X - \mu/\sigma \quad (7)$$

$$z = - (X - \mu/\sigma) \quad (8)$$

z is the score value, X – mean value of the parameter, μ - mean of all samples; σ – standard deviation. Equation (8) was used for parameters where higher values were desired (Hardness, WHC, TDF), and equation (9) was used for formulations where lower values were considered desirable (cook loss, TBARS day 9, emulsion stability). This overall grading system was pictorially represented using a radar chart prepared using the Microsoft Excel Sheet (Microsoft Inc.).

5.2.12 Statistical analysis

The entire study was repeated in three-independent trials (control and treatments) over three days and the replications were as treated as the blocks. The mean ± standard deviation results of the responses presented in this study were obtained by averaging the triplicate values (five in the case of TPA) of all three independent trial values. The single factor impact and the interaction between the US-treated ingredients and different formulations were analysed by two-way ANOVA with US-treated ingredients, formulations and US-treated ingredients*formulations as the factors using Minitab®17.0 statistical software package with means of the data compared using Tukey's comparison ($P<0.05$). In addition, the means of the responses of each formulation (with US-treated AP & CSS) was compared with their respective formulation (with non-US treated AP & CSS) using one-way ANOVA Tukey's comparison ($P<0.05$).

5.3 Results & Discussions

5.3.1 Proximate composition analysis

Proximate composition data for phosphate-reduced sausage formulations are presented in Table 5-1. The proximate content values for each formulation with US-treated AP and CSS along with their respective formulations with non-US treated AP and CSS were compared with each other. As anticipated, results of 2-way ANOVA showed that the inclusion of US-treated AP and CSS in the sausage formulations did not produce a significant interactive impact on the proximate content of sausage formulations. It was noted that the moisture, fat, protein, fibre and salt content values of sausages were nearly the same for all the sausages formulations, irrespective of the AP- and CSS-type used. The values obtained were similar and supported by the validation work conducted in our previous chapter study (See

Chapter 3, Table 3-4, page 84). The ash content of formulations 1 and 2 (1.75-1.80%) with both non-US treated and US-treated formulations was relatively higher when compared with that of formulation 3 (1.64%). This is because of the reduced phosphate concentration and the absence of CSS in formulation 3 since CSS is rich in inorganic minerals such as phosphorus, potassium, calcium, magnesium, iron, sulphur, and others (Ballesteros, Teixeira, & Mussatto, 2014).

5.3.2 Emulsion stability

Emulsion stability is the ability to maintain a food system and structure where the mixing of water and fat in a food product can be accomplished and maintained in a stabilised structure or form over a required timeframe. They indicate unreleased fat and moisture retained by meat proteins (Sarıçoban et al., 2008). The results of the emulsion stability analysis of sausages were expressed using the TEF (%) and fat exudate (%) values, as shown in Table 5-1. In general, the lower the value of TEF (%) and fat exudate (%), the higher the degree of water and fat molecule binding within the meat matrix, thus indicating the higher stability of the emulsions (Yang et al., 2021). The results of 2-way ANOVA showed that US-treatment and the sausage formulations produced a significant interactive effect on TEF (%), however, no such effect was observed for fat exudate (%) values. This indicates that US-treated AP and CSS differently affected the TEF (%) values for different sausage formulations. Statistical data analysis showed that the addition of US-treated AP and CSS to sausage formulations 1 and 3 significantly reduced ($P<0.05$) the values of TEF (%). A similar but insignificant trend was observed for sausage formulations 2. It was also noted that the TEF (%) values for formulations 1 and 2 were low for control (1 – 8.2±0.3%; 2 - 7.5±0.4%) and US-treated samples (1 - 7.7 0.1%; 2 - 7.4±0.2%) when compared with that of the formulation 3 (control – 13.5±0.6%; US – 10.6±0.4%). This could be primarily due to the reduction in the concentration of phosphate (0.06%) used and the absence of CSS in formulation 3. However, there was no such difference in values for fat exudate values between sausage formulations. The statistical one-way ANOVA analysis showed a decreasing trend in fat exudate values (%) for all formulations with US-treated AP and CSS but was found significant only for formulation 1. This decreasing trend in TEF and fat exudate values was mainly due to the increase in the

ingredient's AP and CSS emulsifying capacity when treated with the US. The emulsion stability of formed emulsions depends upon the interfacial protein-membrane and oil-water droplet size (Jiang & Xiong, 2015; Yum et al., 2018). Applying US-led to the partial unfolding of the protein structure, thus exposing the internal hydrophilic and hydrophobic protein groups present in the functional ingredients AP (~ 5% total protein content) and CSS (~18% total protein content). This, in turn, improved the adsorption of oil droplets to the protein structure resulting in their improved emulsifying capacity (Zhang, Liu, Xia, Sun, & Kong, 2021), thus, resulting in increased emulsion stability of phosphate-reduced sausage formulations.

5.3.3 WHC and Cook loss

WHC can be defined as the ability of the meat to maintain its inherent water content when subjected to mincing, cutting, pressing, and also during storage and transport, thereby determining its acceptability, weight loss, cook loss, and sensory traits on consumption (Álvarez, Mullen, Hamill, O'Neill, & Álvarez, 2021; Warner, 2017). Mean values of WHC for all experimental sausage formulations are presented in Figure 5-1. On comparing formulations, it was observed that WHC values of formulations 3 with non-US treated AP and CSS (76.9±1.2%) were lower when compared to formulations 1 (82.8±0.1%) and 2 (82.3±0.7%) with non-US treated AP and CSS. The same trend was observed in sausage formulations with US-treated AP and CSS (1 – 85.0±0.3%; 2 – 85.3±0.6%; 3 – 81.1±0.6%). This observation was due to the reduced phosphate concentration in formulation 3 since phosphates significantly improve WHC in sausages (Glorieux, Goemaere, Steen, & Fraeye, 2017). The results of 2-way ANOVA showed the addition of US-treated AP and CSS to sausage formulations (1 – 85.0 ± 0.3%; 2 – 85.3 ± 0.6%; 3 – 81.1 ± 0.6%) had a significant interactive effect ($P<0.05$) on WHC in sausages. It was observed that WHC values of formulations 2 and 3 with US-treated ingredients significantly increased when compared to their respective formulations with non-US-treated ingredients, which is not the case with formulation 1, where an insignificant increasing trend was observed. This increase in WHC was due to the physical modification induced in the ingredients by the cavitation mechanism produced by the US application. This physical alteration in the ingredient matrix opened the structure of the treated ingredients, thereby increasing

their electric charge and exposing their internally hidden hydrophilic groups to water molecules, resulting in increased water absorption capacity (WAC) of AP and CSS (Téllez-Morales et al., 2020).

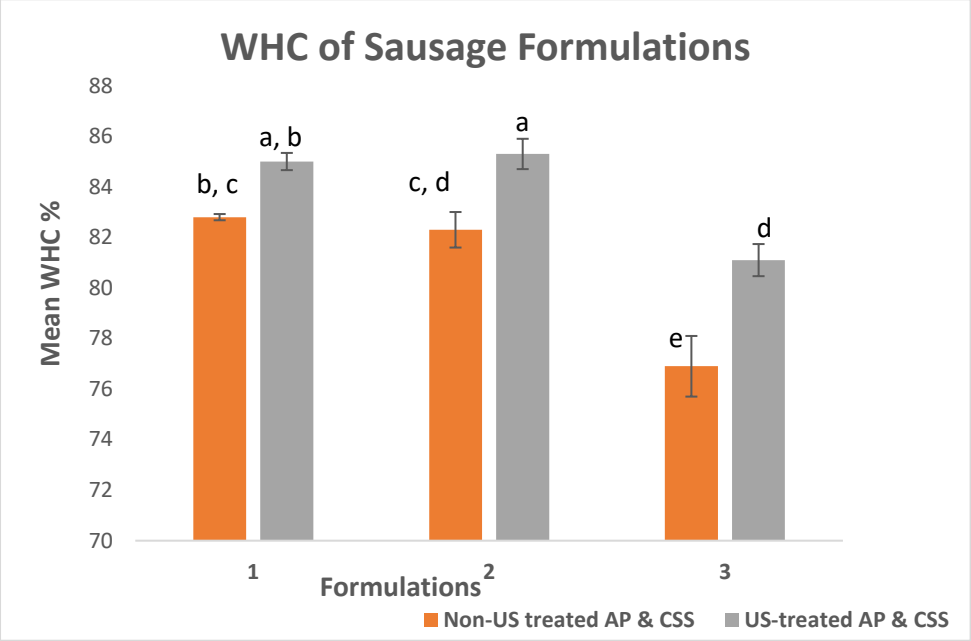


Figure 5-1: Bar chart comparing the WHC of sausage formulations 1, 2 & 3 containing the US-treated AP and CSS with their respective controls containing untreated AP & CSS.

Where Formulation 1- 0.20% STPP+0.22% AP+0.58% CSS; Formulation 2- 0.20% STPP+0.00% AP+ 0.80% CSS; Formulation 3- 0.06% STPP+0.94% AP+0.00% CSS

a-e –Mean values with different superscripts within a chart are statistically different ($P<0.05$) from each other using 2-ways ANOVA followed by Tukey's comparison

Results of 2-way ANOVA showed that change cook loss values were significantly influenced by the interactive effect of sausage formulations and US-treated ingredients. Figure 5-2 showed that introducing US-treated AP and CSS reduced cook loss values of sausage formulations. Statistical analysis showed that this effect was significant ($P<0.05$) for all sausage formulations (formulation 1 (Con – 9.1 ± 0.1%; US – 8.3 ± 0.4%), formulation 2 (Con – 9.1 ± 0.4 %; US – 7.6 ± 0.3 %), formulation 3 (Con -13.1 ± 0.8%; US – 9.6 ± 0.3%)). The reduction in cook loss values can be explained because cook loss is inversely related to WHC (Álvarez et al., 2021). The observed

increase in WHC produced by the tight binding of water molecules resulted in decreased free water molecules in the meat matrix, thereby reducing the cook loss values. It was also observed that cook loss values for formulation 3, irrespective of whether US-treated or non-US treated ingredients were employed, were higher when compared to that of sausage formulations 1 and 2. This is again due to the lower STPP concentration (0.06%) present and the inability of the AP to match up to the STPP level utilised. These analyses showed that the introduction of US-treated AP and CSS improved the quality of phosphate-reduced sausage formulations and positively influenced WHC and cook loss values.

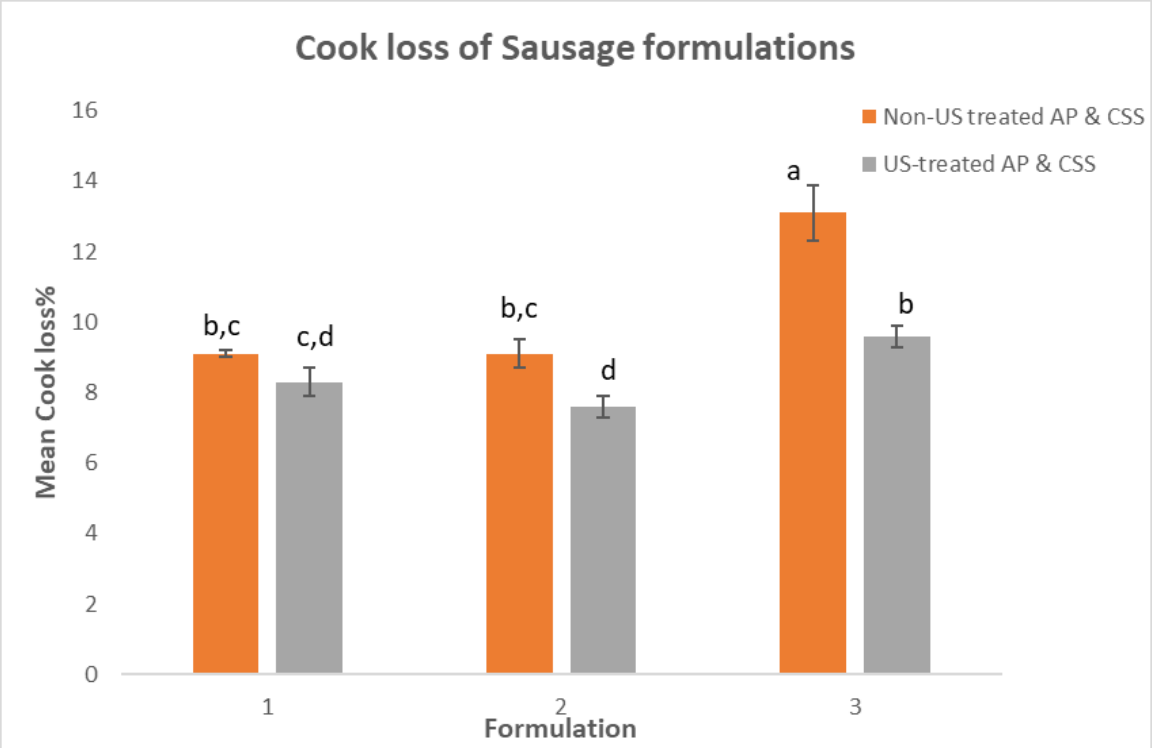


Figure 5-2: Bar chart comparing the cook loss of sausage formulations 1, 2 & 3 containing the US-treated AP and CSS with their respective controls containing untreated AP & CSS

Where Formulation 1- 0.20% STPP+0.22% AP+0.58% CSS; Formulation 2- 0.20% STPP+0.00% AP+ 0.80% CSS; Formulation 3- 0.06% STPP+0.94% AP+0.00% CSS

a-e –Mean values with different superscripts within a chart are statistically different ($P<0.05$) from each other using 2-ways ANOVA followed by Tukey's comparison

5.3.4 Water mobility measured using LF-NMR

LF-NMR relaxation data is generally used to measure water distribution and mobility in the meat muscle matrix; thereby helping in determining various product qualities and attributes such as WHC and drip loss, and sensory attributes (Bertram, Aaslyng, & Andersen, 2005; Li et al., 2012; Pearce, Rosenvold, Andersen, & Hopkins, 2011). The relaxation time distribution T_2 data obtained from LF-NMR analysis can be differentiated into two or three different curve compartments, each representing the various water types in the meat matrix (Bertram, Andersen, & Karlsson, 2001). The first compartment, T_{2b}, with a time constant relaxation of 1-10 ms, represents inner protein-bound water. The second compartment, T_{21}, with a 30-50 ms time constant, represents the myofibrillar active water. The third compartment, T_{22}, with a time constant of 150-400 ms, represents the outer bound or extra-myofibrillar water that contributes to the drip loss. The population distribution under each curve (P_{2b}, P_{21}, P_{22}) demonstrates the amount of water present in the specific relaxation time distribution curve (Bertram et al., 2001; Bertram, Schäfer, Rosenvold, & Andersen, 2004).

Results of relaxation time (T_{2b}, T_{21} and T_{22}) and population distribution (P_{2b}, P_{21} and P_{22}) for sausage samples are presented in Table 5-1. Results of 2-way ANOVA showed that US-treated ingredients and different sausage formulations had significant interactive effect on population distribution values (P_{2b}, P_{21}, P_{22}), which is not the case for relaxation time (T_{2b}, T_{21} and T_{22}). It was observed from one-way ANOVA results that the population distribution of relaxation curve T_{2b} (P_{2b}) of formulations 1 and 2 significantly decreased when US-treated AP and CSS were added. It was reported that the water distributed under the T_{2b} curve was generally unaffected by any mechanical stress (McDonnell et al., 2013), but in our study, changes ($P<0.05$) were observed due to the addition of AP and CSS, confirming our previous chapter results (See Chapter 3, Section 3.3, page 76). This was supported by the significant decrease in the values of T_{2b} for all three formulations when US-treated AP and CSS were added. The values of P_{21} for sausage formulations 1 and 2 showed that adding US-treated AP and CSS increased population distribution ($P<0.05$) compared with

their respective formulations with non-US treated AP and CSS, thereby confirming the increased WHC results observed. However, a decreasing trend in P_{21} values ($P>0.05$) was observed in the case of sausage formulation 3. The results of P_{22} showed that the addition of US-treated AP and CSS reduced values in sausage formulations 1 and 2 that were not significant enough to support the impact of US application. Similar to the results of P_{21}, the P_{22} values of formulation 3 followed an opposite trend compared with that of formulations 1 and 2. This increase in P_{21} and decrease in P_{22} values for formulations 1 and 2 demonstrates that water from outbound the water matrix had moved to the entrapped water matrix, thus contributing to their increased WHC and cook loss values when US-treated AP and CSS were added. However, interestingly, an increase in WHC and a decrease in cook loss values were observed for formulation 3 despite their opposite changing trends in P_{21} and P_{22} values. However, this negative trend in sausage formulation 3, which contained a lower STPP content and higher AP content, can be related directly to their overall lower WHC and higher cook loss values than sausage formulations 1 and 2. This demonstrated that the water mobility in the meat matrix was still majorly influenced by the phosphate concentration. The addition of US-treated AP and CSS to phosphate-reduced sausage formulations positively influenced the water mobility of sausage formulations 1 and 2.

Table 5-1: Mean and standard deviation values of proximate compositions, emulsion stability and LF-NMR population distribution percentage of three independent trials

	Formulation 1			Formulation 2			Formulation 3			Interaction Sig**.
	Non-US treated AP & CSS	US-treated AP & CSS	Sig.*	Non-US treated AP & CSS	US-treated AP & CSS	Sig.*	Non-US treated AP & CSS	US-treated AP & CSS	Sig.*	
Moisture (%)	62.8 ± 0.5	62.3 ± 0.9	ns	62.6 ± 0.5	62.7 ± 0.5	ns	63.7 ± 0.6	62.6 ± 0.9	ns	ns
Fat (%)	13.3 ± 0.1	13.2 ± 0.3	ns	13.5 ± 0.2	13.5 ± 0.4	ns	12.8 ± 0.5	13.5 ± 0.6	ns	ns
Protein (%)	16.0 ± 0.4	16.3 ± 0.7	ns	16.4 ± 0.1	16.0 ± 0.5	ns	16.0 ± 0.5	16.2 ± 0.5	ns	ns
Ash (%)	1.8 ± 0.1	1.8 ± 0.1	ns	1.8 ± 0.0	1.8 ± 0.0	ns	1.6 ± 0.0	1.6 ± 0.1	ns	ns
TDF (%)	7.6 ± 2.7	6.5 ± 4.0	ns	6.2 ± 2.0	7.8 ± 1.9	ns	6.6 ± 2.7	6.8 ± 1.5	ns	ns
Salt (NaCl) (%)	0.7 ± 0.1	0.7 ± 0.2	ns	0.9 ± 0.2	0.8 ± 0.2	ns	0.9 ± 0.2	0.8 ± 0.2	ns	ns
TEF (%)	8.3 ± 0.3^{c}	7.7 ± 0.1^{c}	*	7.5 ± 0.4^{c}	7.4 ± 0.2^{c}	ns	13.5 ± 0.6^{a}	10.6 ± 0.4^{b}	*	**
Fat Exudate (%)	7.4 ± 0.6	5.5 ± 0.2	*	4.3 ± 1.1	4.3 ± 2.9	ns	5.9 ± 1.5	4.4 ± 0.9	ns	ns
T_{2b} (ms)	3.9 ± 0.6	2.5 ± 0.3	*	3.8 ± 0.4	2.4 ± 0.2	*	3.0 ± 0.5	2.1 ± 0.1	*	ns
T_{21} (ms)	36.5 ± 2.1	38.9 ± 0.0	ns	37.7 ± 2.1	37.7 ± 2.1	ns	37.7 ± 2.1	38.9 ± 0.0	ns	ns
T_{22} (ms)	227.3 ± 24.4	257.6 ± 14.1	ns	220.0 ± 21.1	257.6 ± 14.1	ns	234.0 ± 12.8	283.6 ± 15.5	*	ns
P_{2b} (%)	3.4 ± 0.1^{a}	2.4 ± 0.1^{b}	*	3.2 ± 0.4^{a}	2.4 ± 0.1^{b}	*	1.8 ± 0.1^{b}	2.2 ± 0.3^{b}	ns	**
P_{21} (%)	89.9 ± 0.6^{d}	92.1 ± 1.0^{b,c,d}	*	90.3 ± 0.8^{c,d}	92.3 ± 0.1^{a,b,c}	*	94.6 ± 1.2^{a}	93.2 ± 1.2^{a,b}	ns	**
P_{22} (%)	6.8 ± 0.6^{a}	5.5 ± 1.0^{a,b,c}	ns	6.5 ± 0.8^{a,b}	5.3 ± 0.1^{a,b,c}	ns	3.5 ± 1.0^{b,c}	4.5 ± 0.9^{c}	ns	**

Where Formulation 1- 0.20% STPP+0.22% AP+0.58% CSS; Formulation 2- 0.20% STPP+0.00% AP+ 0.80% CSS; Formulation 3- 0.06% STPP+0.94% AP+0.00% CSS

*- Significance level at $P < 0.05$ comparing the effect of US-treated AP and CSS between the individual sausage formulations, ns- not significant

**- Interaction significance levels at $P < 0.05$ of the relationship between US treatment and formulations using 2-way ANOVA, ns- not significant

a-d - Mean values with different superscripts within a row are statistically different ($P<0.05$) from each other using Two-ways ANOVA followed by Tukey's comparison

5.3.5 Colour

The instrumental colour analysis results of raw sausage formulations are presented in Table 5-2. Based on the previous chapter study, significant changes in the colour parameters of AP and CSS powders were observed when they were treated with the US for 30 mins (See Chapter 4, Section 3.5, page 106). However, when integrated into a sausage batter, according to the one-way ANOVA results, US-treated AP and CSS produced no significant changes in the mean L*and b* values of the sausage formulations compared to their respective formulation with non-US treated AP and CSS. The introduction of US-treated AP and CSS had increased a* values, but this was insignificant, except for formulation 2, where significant ($P<0.05$) changes in a* values were observed. This increase in the sausage redness was due to the colour change produced in the AP and CSS owing to US-treatment and freeze-drying. In addition, results of 2-way ANOVA showed that US-treated ingredients and formulations did not produce a significant interaction on the colour values. The readings of ΔE measuring the visual colour difference between formulations with non-US treated ingredients and their respective sausage formulations containing US-treated AP and CSS were 0.82, 0.60 and 0.89. Based on Mokrzycki & Tatol (2011), no colour differences will be observed between samples if samples possess values ΔE between $0 < \Delta E > 1$ and a visible colour difference will be observed for inexperienced observers if values of over $\Delta E > 2$ are achieved. Thus, no visible colour differences were observed between the sausage formulations with US-treated ingredients and their respective opposites. When compared within formulations with the US-treated AP and CSS, formulation 1 and 2 was observed to have lower L* values (1- 61.3±1.8; 2 – 59.9±2.2) compared with formulation 3 (64.1±1.0). Similar observations were found in formulations 1, 2 and 3 with non-US treated AP and CSS. The lower L* values of formulations 1 and 2 were due to CSS's presence. This is similar to Martuscelli et al. (2021), where adding CSS increased the darkness of the chicken meat burgers.

Table 5-2: Mean and standard deviation values of colour analysis, textural parameters and TBARS values of days 0, 3, 6 and 9 of three independent trials

		Formulation 1			Formulation 2			Formulation 3			Interaction Sig.**
		Non-US treated AP & CSS	US-treated AP & CSS	Sig.*	Non-US treated AP & CSS	US-treated AP & CSS	Sig.*	Non-US treated AP & CSS	US-treated AP & CSS	Sig.*	
L*		61.8 ± 2.0	61.3 ± 1.8	ns	59.9 ± 1.5	59.9 ± 2.1	ns	64.9 ± 2.2	64.1 ± 1.0	ns	ns
a*		6.3 ± 0.2	6.6 ± 0.4	ns	6.5 ± 0.1	6.9 ± 0.2	*	6.3 ± 0.4	6.5 ± 0.2	ns	ns
b*		18.4 ± 1.3	18.9 ± 1.3	ns	18.9 ± 0.7	19.4 ± 0.8	ns	20.1 ± 1.3	20.1 ± 0.6	ns	ns
ΔE			0.82			0.60			0.89		
Hardness (N)		26.6 ± 5.0	27.7 ± 2.6	ns	27.4 ± 8.4	27.2 ± 3.7	ns	20.7 ± 1.7	20.8 ± 3.2	ns	ns
Chewiness (J)		40.2 ± 21.8	31.4 ± 6.9	ns	38.0 ± 21.6	33.7 ± 14.7	ns	14.2 ± 1.0	13.0 ± 2.8	ns	ns
Cohesive force (no unit)		0.7 ± 0.0	0.7 ± 0.0	ns	0.8 ± 0.0	0.8 ± 0.0	ns	0.8 ± 0.0	0.8 ± 0.0	ns	ns
Gumminess (N)		6.0 ± 3.0	4.9 ± 0.9	ns	5.5 ± 2.7	5.3 ± 1.6	ns	3.2 ± 0.3	3.0 ± 0.4	ns	ns
Springiness (mm)		6.6 ± 0.3	6.4 ± 0.3	ns	6.7 ± 0.9	6.1 ± 0.8	ns	4.5 ± 0.1	4.4 ± 0.3	ns	ns
TBARS (mg MDA/kg)	**day 0**	0.2 ± 0.0	0.2 ± 0.0	ns	0.2 ± 0.0	0.2 ± 0.0	ns	0.2 ± 0.0	0.2 ± 0.0	ns	ns
	day 3	0.2 ± 0.0	0.2 ± 0.0	ns	0.2 ± 0.0	0.3 ± 0.1	ns	0.2 ± 0.0	0.3 ± 0.0	ns	ns
	day 6	0.3 ± 0.1	0.3 ± 0.1	ns	0.3 ± 0.1	0.3 ± 0.1	ns	0.4 ± 0.2	0.4 ± 0.1	ns	ns
	day 9	$0.3 \pm 0.0^{b,c}$	0.4 ± 0.0^{b}	ns	0.3 ± 0.0^{c}	0.4 ± 0.0^{b}	*	0.4 ± 0.1^{b}	0.8 ± 0.1^{a}	*	**

Where Formulation 1- 0.20% STPP+0.22% AP+0.58% CSS; Formulation 2- 0.20% STPP+0.00% AP+ 0.80% CSS; Formulation 3- 0.06% STPP+0.94% AP+0.00% CSS

*- Significance level at $P < 0.05$ comparing the effect of US-treated AP and CSS between the individual sausage formulations, ns- not significant

**- Interaction significance levels at $P < 0.05$ of the relationship between US treatment and formulations using 2-way ANOVA, ns- not significant

a-c - Mean values with different superscripts within a row are statistically different ($P<0.05$) from each other using Two-ways ANOVA followed by Tukey's comparison

5.3.6 Texture profile analysis (TPA)

Results of texture parameters for sausage formulations consisting of hardness, gumminess, springiness, chewiness and cohesive force values are presented in Table 5-2. Results of 2-ways ANOVA analysis of texture parameters showed no significant interactive differences produced between the sausage formulations and US-treated AP and CSS. A decreasing values trend was observed for chewiness, gumminess and springiness for all formulations comprised of US-treated AP and CSS compared with their respective opposite formulations. However, these changes were insignificant ($P>0.05$). In the previous Chapter (See Chapter 3, Section 3.5, page 79), the authors showed that the increase in AP and CSS concentration reduced the hardness and chewiness values in sausage formulations containing higher STPP. Thus, the introduction of US-treated AP and CSS to improve the textural characteristics of phosphate-reduced sausage formulations did not produce any significant changes. Although there was a significant increase in WHC and emulsion stability in sausages formulated with US-treated AP and CSS, the data generated did not influence sausage textural properties. Additionally, and similar to other functional properties, sausage formulation 3, with lower phosphate concentration (0.06%), recorded lower textural values compared with phosphate-reduced sausage formulations 1 and 2 (0.20%) in terms of hardness, chewiness, and gumminess and springiness values. This reduction in sausage textural properties from formulation 3 decreased its firmness, affecting its potential eating quality. This finding demonstrates that sausage texture is still primarily dependent on STPP concentration, despite US-treated AP and CSS employment in sausage formulations.

5.3.7 Lipid oxidation of sausages

Lipid oxidation measurements in sausage formulations were expressed in milligrams of malondialdehyde produced per kilogram of sausage (Table 5-2) using the TBARS method. Results of 2-way ANOVA, measuring the interaction effect between the US-treatment and the formulations, did not produce any significant interaction effect on TBARS values on day 0, 3 and 6. However, the effect was significant ($P<0.05$) for TBARS value on day 9 indicating that the US-treated ingredients produced different effect on different formulations. Results of one-way ANOVA showed that TBARS

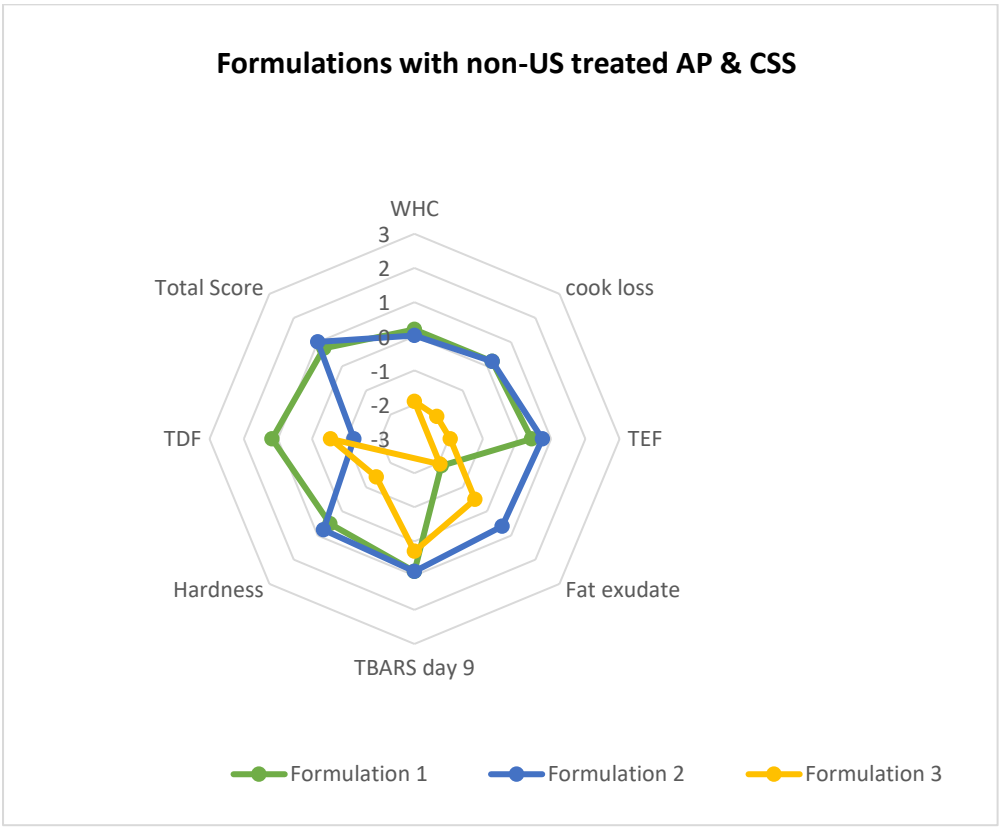
values (measured on storage days 0, 3 and 6) of sausage formulations containing US-treated ingredients were not significantly different from their respective formulations with non-US treated ingredients. However, in case of day 9 TBARS values, it was evident that sausages containing US-treated AP and CSS had higher ($P<0.05$) values for formulations 2 and 3 ($P<0.05$) when compared with their respective formulations with non-US treated ingredients, with the exception being determined for formulation 1. However, the increase in TBARS value ranges were well within the detectable threshold limit of 2.0-2.5mg MDA/kg (Zhang et al., 2019), the highest upper range where no off-flavours are expected to appear, and the formation of dangerous free radicals was in control. Comparing the formulations in general, it was observed that sausage formulations 1 and 2 had lesser day 9 TBARS values when compared to formulation 3, which was expected because of the lower STPP concentration (0.06%) of formulation 3. This may be explained by the fact that phosphates are known to be excellent antioxidants (See Chapter 1, Section 4, page 11). However, this was not observed for TBARS values measured on day 0, 3, and 6. This control in TBARS values was due to the addition of AP and CSS which are very good antioxidants even after the US treatment. Therefore, the addition of US-treated AP and CSS to improve the quality of phosphate-reduced sausage formulations resulted in the increased value of lipid oxidation on day 9 analysis.

5.3.8 Sausage formulation scoring

The results of the sausage scoring system employing important physicochemical product properties are shown in Figure 5-3 (i &ii). The scores of phosphate-reduced sausage formulations improved with the US-treated AP and CSS compared to the addition of non-US treated AP and CSS. The total scores (divided by 2) of sausage formulations with non-US treated and US-treated AP and CSS, respectively, were as follows: (i) Formulation 1 – 0.75 & 1.29; (ii) Formulation 2 – 1.00 & 2.93; and (iii) Formulation 3 – (-) 4.05 & (-) 1.94, indicating the quality improvement in sausage formulations by the addition of US-treated AP and CSS. It was noted that tenderness and TBARS day-9 values had more weight on these scores, along with WHC, cook loss and emulsion stability. The TDF can also be validated as a more important trait with reduced-phosphate content. Although both AP and CSS contain almost the same TDF

%, the concentration of STPP added and their interaction with STPP produced differences in formulation scores. On reflection, the scores of formulation 2 containing 0.80% CSS and 0.20% STPP had the highest TDF values, whereas formulation 3 containing 0.94% AP and 0.06% STPP recorded the lowest TDF values. In summary, STPP concentration still had an influential impact on TDF scores, and thus, formulation 2, which possessed the higher score, can be regarded as the best sausage formulation. The scores also showed that CSS had a better phosphate-reducing capability when compared to AP.

(i)



(ii)

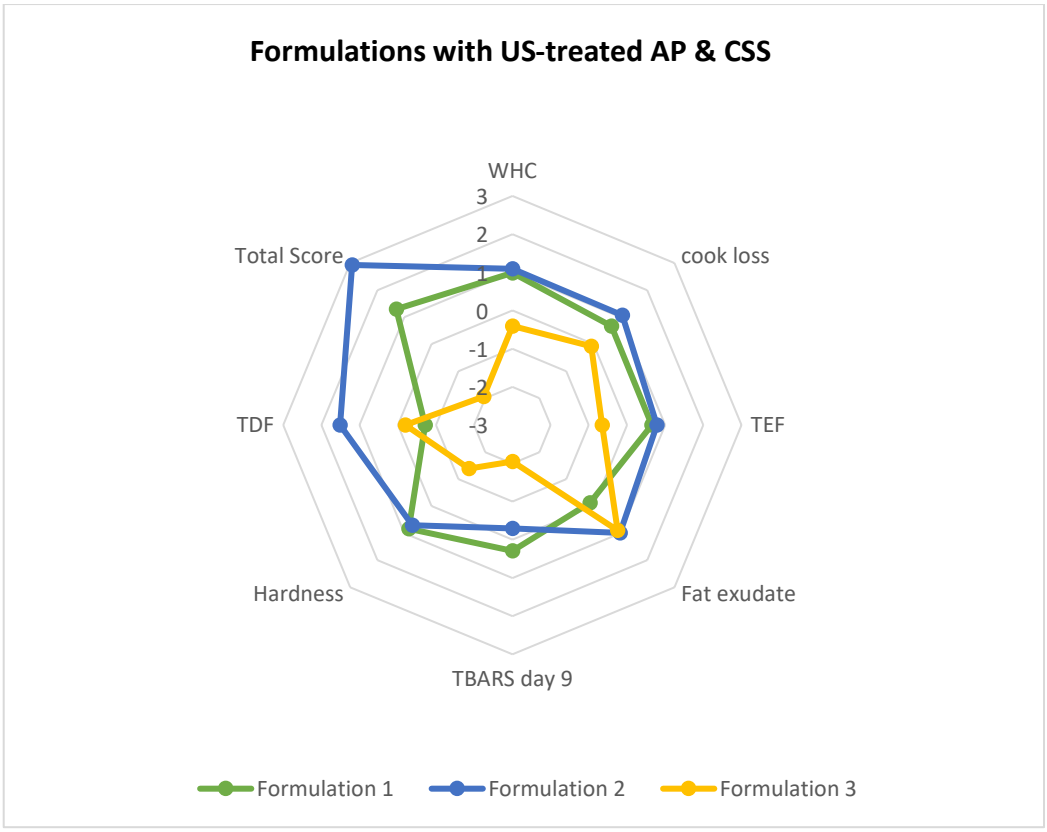


Figure 5-3: Scores for each of the main physicochemical properties of sausage formulations 1-3. (i) Non-US treated AP & CSS in sausage formulations (ii) US-treated AP & CSS in phosphate-reduced sausage formulations. Total score for each treatment (divided by 2).

Where Formulation 1- 0.20% STPP+0.22% AP+0.58% CSS; Formulation 2- 0.20% STPP+0.00% AP+ 0.80% CSS; Formulation 3- 0.06% STPP+0.94% AP+0.00% CSS

5.4 Conclusion

The present study successfully investigated the ability of US-treated AP and CSS to improve the physicochemical properties of phosphate-reduced Irish breakfast sausages formulations. Interpretation of the significant sausage properties influenced by phosphates such as WHC, cook loss and emulsion stability showed that US-treated AP and CSS positively affected phosphate-reduced sausages compared to those generated using non-US treated AP and CSS, improving their quality. In addition, changes in these properties were significantly influenced by the interactive relationship effect produced by the addition of US-treated ingredients and different sausage formulation, meaning that different formulations reacted differently to the

addition of US-treated AP and CSS. Findings from this study clearly showed that STPP could be reduced from 0.5% to 0.2% with improved product quality attributes when US-treated AP and CSS are added to Irish breakfast sausage formulations. Further analysis showed that formulation 2, containing only CSS as an STPP replacer, had better physicochemical properties than sausage formulation 3, which contained only AP. Thus, US-treated CSS is the best STPP-replacer compared to AP. However, increased TBARS values were the study's primary concern, although the values observed were within acceptable thresholds as determined scientifically.

Chapter 6. Effect of high-pressure processing in improving the quality of phosphate-reduced Irish breakfast sausages formulated with ultrasound-treated phosphate alternatives

Overview

This chapter examines the combined effect of high-pressure processing (HPP) treated pork meat and US-treated apple pomace (AP) and coffee silverskin (CSS) on the quality characteristics of phosphate-reduced sausage formulations obtained from Chapter 3. The physicochemical properties of sausage formulations were compared with that of control sausage formulations to assess the quality improvement produced by HPP and US. The best formulation in terms of quality was evaluated using the scoring system. A manuscript has been submitted to peer review journal for publication from this chapter.

- **Thangavelu, K. P.,** Tiwari, B. K., Kerry, J. P., & Álvarez, C. Effect of high-pressure processing in improving the quality of phosphate reduced Irish breakfast sausage replaced with ultrasound treated phosphate alternatives. *Meat Science, 194,* 108981.

6.1 Introduction

Global food processing industries have undergone tremendous changes in recent years concerning approaches employed in processing food products to meet the ever-changing requirements of consumers. Of particular note, consumer realisation of the association between food and health has resulted in a rapid increase in consumer demand for natural, higher quality, nutritious, and healthy food products that are free from any added preservatives or additives (Barbut, Wood, & Marangoni, 2016; Hygreeva & Pandey, 2016) and all delivered sustainably. The meat industry is one of the major food processing sectors and has been hugely affected by this consumer demand (Soladoye, Pietrasik, Hrynets, & Betti, 2021).

For centuries, the consumption of meat and processed meat products has been a part of human evolution (Moreira, da Veiga, da Veiga, Reis, & Pascuci, 2022). However, in recent years, and due to the growing consumer trends described above, there are predominant health concerns over the general consumption of processed meat products. Consumption of traditional processed meat products has contributed to the higher intake of fat, salt, and synthetic additives, primarily phosphates, resulting in various associated health issues such as high blood pressure and cardiovascular and heart diseases (Conroy, O'Sullivan, Hamill, & Kerry, 2018). It was reported in the National adult nutrition survey that sausage was consumed by 39% of the Irish population of age 18-64 years and 31% of those of age ≥65 years (Irish Universities Nutrition Alliance IUNA, 2011; Conroy et al., 2018). This higher consumption has resulted in reducing and/or replacing various additives added to breakfast sausage by meat industries. Phosphate reduction in breakfast sausages is one such consumer-driven challenge currently faced by meat industries (O'Flynn, Cruz-Romero, Troy, Mullen, & Kerry, 2014). Phosphates are synthetic additives, generally added to processed meat such as sausages to bind added water molecules, elevate pH, stabilise meat emulsions, reduce oxidative rancidity, improve juiciness, tenderness, appearance, firmness and maintenance of product flavour (Long, Gál, & Buňka, 2011; O'Flynn et al., 2014). Higher consumption of phosphates can cause Hyperphosphatemia (higher accumulated blood phosphates) in people with chronic kidney diseases, thereby increasing their rate of mortality up to 40%, and can also

reduce the absorption of calcium into bones within healthy individuals (Pinton et al., 2021; Takeda, Yamamoto, Yamanaka-Okumura, & Taketani, 2014). This signifies the importance of phosphate removal or control from sausages for the benefit of the general population, but specifically for population groups impacted by the presence of phosphates in the diet. However, alteration or removal of phosphate from processed meat products is a massive challenge for processed meat industries because of the critical roles played by phosphates in meat processing. Attempting to remove phosphates without compromising the eating quality of processed meat products is complex and very easily detected by consumers. This change in quality can be counteracted by the introduction of natural functional food ingredients as phosphate alternatives or by using advanced novel, non-thermal food processing technologies such as; power ultrasound (US), high-pressure processing (HPP), oscillating magnetic fields and cold plasma technologies to produce high quality and minimally-processed food (Khouryieh, 2021). Combining both approaches opens up the number of possibilities that can be employed to replace, reduce or control phosphate levels in relevant processed meat systems and products. In this work, US and HPP were combined at different points of the sausage manufacturing process, the objective being to improve final product quality. US application was employed to treat phosphate-replacing ingredients. HPP was used to treat the fresh pork meat following mincing because of the technology's proven ability to enhance ingredient performance and modify muscle properties, discussed in more detail below.

The US is a green, non-thermal food processing technology that functions by creating vapour bubbles in liquid, solid, dispersion or gaseous media produced by exposing them to sound waves of higher frequencies (> 20kHz)(Arzeni et al., 2012; Cichoski et al., 2019). The US can be used to effectively modify the structure and improve ingredient functionalities (Pinton et al., 2019). More specifically, apple pomace (AP), obtained from the apple juice industry, and Coffee silver skin (CSS), obtained from the coffee powder industry, were the two functional food co-products or ingredients of interest used as phosphate alternatives in this study. These ingredients are two dietary, fibre-rich, techno-functional ingredients which contain natural phosphorous levels of 1.4g/kg (AP) and 1.5g/kg (CSS), respectively (See Chapter 3, Section 1, page

62; Martuscelli, Esposito, Di Mattia, Ricci, & Mastrocola, 2021). The presence of higher contents of total dietary fibre (TDF), mainly insoluble fibre, in AP (78-90%) and CSS (86%) can be used to improve the WHC, emulsion stability, cook loss and textural characteristics when introduced into meat products (Illippangama, Jayasena, Jo, & Mudannayake, 2022). The previous chapter (See Chapter 3, Section 3.7, page 82) demonstrated CSS and AP's phosphate-replacing/reducing capabilities using a mixture design approach, which developed three optimised phosphate-reduced sausage formulations. These three formulations were later assessed following US treatment to ascertain if AP and CSS could be further improved concerning their functionalities and product quality improvements. Data generated from this study showed that the inclusion of US-treated (250W, 20 kHz for 30 mins) AP and CSS in phosphate-reduced Irish breakfast sausages formulations improved their physicochemical properties, such as; WHC, cook loss and emulsion stability (See Chapter 5, Section 4, page 130). However, all three formulations detected no significant improvements in textural product attributes or oxidative inhibition values. Hence, in this study, we explored the possibilities of further improving the quality of these optimised sausage formulations using HPP.

HPP is the process of applying high hydrostatic pressure (100-800 MPa) evenly on packaged liquid or solid food surfaces for milliseconds to several minutes at refrigeration or mild temperatures (<45^{o}C) using a liquid medium for pressure transfer (Hernández-Hernández, Moreno-Vilet, & Villanueva-Rodríguez, 2019; Khouryieh, 2021; Muntean et al., 2016). HPP food application modifies food structure, denatures proteins and inactivates microorganisms and enzymes. These structural changes increase mass transfer rates, solvent permeability and secondary metabolite diffusion within the food (Andreou et al., 2017). HPP has emerged over the past two decades as a fast-growing eco-friendly, non-thermal food processing technology that offers adequate safety and quality advantages to meat and processed meat products (Baptista, Rocha, Cunha, Saraiva, & Almeida, 2016; Xue et al., 2017). Grossi et al. (2016) reported that HPP, when employed at moderate pressures (100-300 MPa), can effectively modify meat protein without excessive denaturation, resulting in improved physicochemical properties like water holding

capacity (WHC), texture and rheological properties. They can be used to produce minimally processed, additive-free meat products (O'Neill et al., 2018). For example, a study by O'Neill et al. (2019) to develop low-salt, shelf-stable frankfurters using the response surface methodology approach showed that a significant salt reduction could be achieved with salt replacer (0–100%), high-pressure processing (HPP) (0.1–600 MPa) and a mix of organic acids (0.2–0.4%). Similarly, O'Flynn et al. (2014) showed that HPP employed at 150 MPa (5 mins) successfully reduced phosphate concentration in breakfast sausages to 0.25%, without any significant changes in their physicochemical properties.

Although individual reports for the US and HPP application to reduce phosphate in meat products have been published, no information on the combined effect of US and HPP on phosphate-reduced sausage manufacture employing AP and CSS as phosphate replacing ingredients could be determined from an extensive review of scientific literature. Thus, the study's main objective was to treat the alternative ingredients AP and CSS with the US and to study its combined effect with HPP-treated pork in improving the physicochemical properties of phosphate-reduced sausage formulations.

6.2 Methods and Materials

6.2.1 Pre-treatment of ingredients and meat

The alternative natural ingredients CSS and AP were supplied by Illy S.P. A. (Trieste, Italy) and Muns Agroindustrial S. LO. (Lleida, Spain), respectively. The individual aqueous solution (10% w/v) of the oven-dried (40^{o}C) and finely-powdered ingredients were treated with high power (250W, 20kHz) US probe (UIP1000hDT, Hielscher Ultrasound Technology, Germany) for 30 mins in temperature-controlled (≤20^{o}C) jacketed glass beakers. Treated solutions were dried to a powdered form using a freeze drier (Cuddon Freeze Dry, New Zealand) and stored (4^{o}C) in airtight containers until further use. The above-chosen parameters for US treatment were based on results from our previous chapter (See Chapter 4, Section 4, page 107), which showed that ingredients treated with the US for 30 mins had improved characteristics compared to those treated for 15 mins.

Four fresh pork loins were used for each experimental repetition (Table 6-1) (pH 5.3–6.0), and all pork loins were derived from the same production batch and were purchased from a local butcher (Gleeson Butchers, Dublin, Ireland) all used in for sausage production. Loin back-fat was trimmed, and the lean meat loins were cut into two halves. A randomly selected loin half was minced together using a meat mincer (Meat Grinder MG510, Kenwood, UK) and vacuum packaged before treating them with HPP. The remaining untreated loin halves were minced together and used to manufacture control sausages.

Table 6-1: The split-plot experimental design of independent trials in terms of HPP treatment and formulations

Repetition	HPP treatment	Formulations
1	HPP	Control
1	Non-HPP	F1
1	HPP	F1
1	Non-HPP	F3
1	Non-HPP	Control
1	HPP	F2
1	HPP	F3
1	Non-HPP	F2
2	HPP	F3
2	Non-HPP	F2
2	Non-HPP	Control
2	HPP	Control
2	HPP	F2
2	Non-HPP	F1
2	Non-HPP	F3
2	HPP	F1

Where Control – 0.50% STPP only; Formulation 1 (F1) - 0.20% STPP+0.22% AP+0.58% CSS; Formulation 2 (F2) - 0.20% STPP+0.00% AP+ 0.80% CSS; Formulation 3 (F3) - 0.06% STPP+0.94% AP+0.00% CSS

The packaged minced meat was treated with HPP at 150 MPa for 5 mins using HPP technology (Stansted Fluid Power Ltd., Essex, UK) containing a mixture of water-oil (90:10) as transmitting fluid (600 MPa as maximum working pressure capacity). The above pressure parameters were chosen based on O'Flynn et al. (2014) report. They showed that treating pork meat at 150 MPa for 5 min improved textural properties and did not negatively affect other properties of breakfast sausages with reduced phosphate (0.25%), whereas 300 MPa (5 mins) negatively affected sausage quality.

Pre- and post-treatment temperatures for samples were maintained between 6.5-8.5°C. HPP-treated meats were immediately used for sausage production.

6.2.2 Sample preparation

Phosphate-reduced sausages were produced by adding US-treated AP and CSS in three optimised mixture formulations obtained from the validation study (See Chapter 3, Section 3.7, page 82) and a control formulation with 0.5% w/w phosphate. The seasoning mix, containing no phosphates, was purchased from Redbrook ingredients (Dublin, Ireland), while rusk and STPP required for sausage manufacture were bought from All in All ingredients (Dublin, Ireland). Control sausage formulations were produced containing untreated/HPP-treated pork meat (58.00%), fat (20.85%), water/ice (13.45%), rusk (5.75%), seasoning (1.45%) and STPP (0.50%). In contrast, the other three mixture formulations containing fat (20.4%) and ingredient mixture formulations of STPP, US-treated AP and CSS (1.00%) were produced. The mixture formulations were as follows,

(i) Formulation 1 - 0.20% STPP + 0.22% AP + 0.58% CSS

(ii) Formulation 2 - 0.20% STPP + 0.00% AP + 0.80% CSS

(iii) Formulation 3 - 0.06% STPP + 0.94% AP + 0.00% CSS

A total of 20 sausages (~10cm in length; 23 mm diameter) per formulation and replication were prepared, and sausages were chosen randomly for further physicochemical analyses. The prepared sausages were packed in a padded black food packaging tray (h 197mm x w 155mm x d 30mm; Silverstream packaging Ltd, Cork, Ireland) overwrapped using polyvinylidene chloride (PVDC) wraps (gas permeability−2.5 [g 100µm]/[m2d]; 300mm x 300m, Prowarp, Bristol, UK), and stored under simulated retail chilled conditions (EXPO PT, glass door upright display cooler, Framec, Italy) at 3-5°C throughout the analysis. The effect of HPP-treated meat on physicochemical properties from all three sausage formulations and the control formulation was studied by comparing them with those sausage formulations containing non-HPP treated meat. The physicochemical properties of breakfast sausages were WHC, cook loss, emulsion stability, texture profile analysis (TPA), lipid oxidation, colour, and proximate composition. Two independent trials were carried out on two different occasions.

6.2.3 Physicochemical properties

Three prepared sausage batters per formulation were assessed for quality alterations: WHC, cook loss and emulsion stability following HPP treatment. WHC and cook loss for sausages were evaluated using the method of Lianji & Chen (1989) with minor modifications described in Chapter 3, Section 2.2, page 64. The alteration in sausage WHC produced by HPP application was extensively studied using LF-NMR analysis. The activity of bound, myofibrillar and free water within the meat matrix was analysed based on the method described by McDonnell et al. (2013) using an LF-NMR Ultra instrument (Oxford instruments, Abington, Oxfordshire, UK) at a resonating frequency of 23.2 MHz.

Sausage batter stability was evaluated by centrifuging raw batter (~25g; exact weight recorded) at 2958g (1 min) in a 50ml centrifuge tube followed by heating at 70^{o}C (30min) in a water bath. Tubes were centrifuged again at 2958g (3 mins), and the supernatants were poured into pre-weighed crucibles for overnight drying at 100^{o}C. The dried pellets were weighted to measure the volume of total expressible fluids (TEF (%)) and fat exudate (%) using the formula reported by Hughes, Mullen, and Troy (1998) as follows:

TEF = Weight of sample – weight of pellet (1)

TEF (%) = (TEF/ sample weight) x 100 (2)

Fat Exudate (%) = (Dried supernatant/ TEF) x 100 (3)

6.2.4 TBARS method for oxidation evaluation

As determined over storage time, lipid oxidation levels in sausages were measured by the TBARS analysis method (Botsoglou et al., 1994), with some modifications described in Chapter 3, Section 2.5, page 66. Results were expressed as milligrams of malondialdehyde produced per kg of sausage (mg MDA/kg). Three sausage samples from day 0 and day 9 per formulation were analysed.

6.2.5 Colour analysis

Sausages covered in transparent PVDC cling film were analysed for changes in colour parameters impacted by HPP treatment. The three colour parameters L* (lightness),

a* (redness) and b* (yellowness), were measured using the dual xenon flash UltraScan Pro spectrometer (Hunterlab, Reston, VA, USA), calibrated by a light trap (L=0) and a transparent cling film covered standard white tile (L=100; X=88.69; Y=93.58; Z=100.45). The viewing port was 25.54mm, and the standard illuminant D65 was used with an observer angle of ten degrees. Sausages were measured in triplicates per formulation and averaged for statistical analysis. The total colour difference between formulations was calculated using the equation presented by Salgado, Fernández, Drago, and Mauri (2011).

$$\Delta E^*_{ab} = \sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2} \quad (4)$$

6.2.6 Textural properties

The major textural parameters for sausages, such as; hardness (N), chewiness (N), gumminess (N), springiness (mm) and cohesion force ratio, were measured based on the methods of Bourne (1978). Five uncooked sausages per formulation and per replication were cooked all together in a single batch were cooked individually in a water bath (73±1°C) for 20-30 mins until a sausage core of 70°C was achieved and then cooled overnight in a refrigerator at 4°C. Cooked sausage TPA values were calculated using the two-cycle compression test applied by an Instron universal testing machine, model 5534 (Instron Ltd., High Wycombe, UK). Sausage dimension cores of 14mm diam. X 20mm ht. was axially compressed at 70% of their original height at a crosshead moving speed of 100mm/min and a 500N load cell, and this was used to determine the force-time deformation curves. Average values of five cores per formulation were recorded.

6.2.7 Component analysis

The proximate composition of the homogenised sausages per formulation was measured using their respective AOAC methods, such as; protein (AOAC 992.15, 1992), moisture (AOAC 985.14, 1990), fat (AOAC 2008.06, 2008), ash (AOAC 920.153, 1920), salt (AOAC 935.47, 1987) and TDF (AOAC 991.43, 1995). The values were measured in triplicate per independent trial and averaged for statistical analysis.

6.2.8 Formulation Grading system

To determine the best overall formulation that can replicate the control formulation, considering all important parameters, a grading system was developed based on the study of Álvarez, Drummond, & Mullen (2018). Each parameter was standardised using the equations,

$$z = X - \mu/\sigma \quad (5)$$

$$z = - (X - \mu/\sigma) \quad (6)$$

where z is the score value, X is the parameter's value, μ - mean of all samples; σ – standard deviation. Equation (5) was used for parameters where higher values are desirable (e.g. WHC), and equation (6) was used for parameters where lower values were desired (e.g. Cook loss). A radar chart was prepared using the Microsoft Excel Sheet (Microsoft Inc.) to represent the overall grading system using the above equations pictorially.

6.2.9 Statistical evaluation

This randomised study was built using a split-plot experimental design, with HPP/Non-HPP treatment as the main effect to be compared within each formulation on the whole plot and one of the four formulations (including control formulation) on the subplot in Table 6-1 and the frequency distribution in Table 6-2. The experiment was replicated twice on two different occasions, and the replications were treated as the blocks to account for any difference between replications. The mean values for the responses represented were the average values of triplicates obtained from both independent study replications (n=6). The single factor impact and the interaction effect between the HPP treatment and different formulations was analysed by two–way analysis of variance (ANOVA) with HPP treatment, formulations and HPP treatment*formulations as the factors using Minitab®17.0 statistical software package. Tukey's multiple comparisons were used to compare the means of data with a confidence interval of 95%. In addition, one-way ANOVA, along with Tukey's multiple comparison, was carried out between control (traditional sausage formulation) with non-HPP treated meat and sausage formulations (1-3) with HPP-treated meat to compare their mean values.

Table 6-2: The frequency distribution of the two independent trials of the study

Frequency	Table of HPP by Formulations					
	HPP Treatment	Formulations				
		Control	F1	F2	F3	Total
	HPP	2	2	2	2	8
	Non-HPP	2	2	2	2	8
	Total	4	4	4	4	16

Where Control – 0.50% STPP only; Formulation 1 (F1) - 0.20% STPP+0.22% AP+0.58% CSS; Formulation 2 (F2) - 0.20% STPP+0.00% AP+ 0.80% CSS; Formulation 3 (F3) - 0.06% STPP+0.94% AP+0.00% CSS

6.3 Results & Discussion

6.3.1 Compositional analyses of sausages

The results of various analyses determining the proximate composition of the raw sausages are presented in Table 6-3. The results of 2-way ANOVA showed that HPP application did not produce any significant interaction in compositional changes of sausage formulations concerning moisture, protein, fat, ash, and salt percentages. Additionally, from the results of one-way ANOVA, it was observed that phosphate-reduced formulations with HPP-treated meat did not affect proximate composition compared to the control formulation with non-HPP treated meat containing 0.50% STPP. For instance, moisture, fat, protein, and salt content of formulations 1, 2 and 3 with HPP-treated meat were almost the same as control formulations (moisture 61.75–62.84%; fat–14.46-15.13%; protein-16.12–16.58%; salt–0.66-0.81%). However, a significant difference in ash and TDF values was observed for the sausage formulations with HPP-treated meat compared with the control with non-HPP treated meat. Sausage formulations 1, 2 and 3 had increased ($P<0.05$) TDF values due to AP and CSS, which are both rich in fibre content. The presence of inorganic STPP in the control (0.50%) and formulations 1 (0.20%) and 2 (0.20%) resulted in higher ash contents ($P<0.05$) when compared to formulation 3 (0.06%). The slightly higher ash values of sausage formulations 1 and 2 compared to formulation 3 were also due to higher mineral compositions present in CSS (Ballesteros, Teixeira, & Mussatto, 2014).

Table 6-3: Mean and standard error mean values of compositional analysis, emulsion stability (as TEF% and fat exudate %), cook loss and NMR population distribution percentage of sausage formulations

	Control		Formulation 1		Formulation 2		Formulation 3		Interaction Sig.	SEM
	Non-HPP	HPP	Non-HPP	HPP	Non-HPP	HPP	Non-HPP	HPP		
Moisture (%)	62.5	61.9	61.9	61.7	62.1	61.9	62.1	62.8	ns	0.1
Fat (%)	14.8	15.3	15.0	15.0	15.1	15.1	15.0	14.4	ns	0.1
Protein (%)	16.1	15.7	16.3	16.3	16.5	16.5	16.1	16.4	ns	0.1
Ash (%)	2.1	2.1	1.9	1.9	1.9	1.9	1.9	1.8	ns	0.0
TDF (%)	0.8	1.5	4.1	3.6	3.5	4.2	4.4	4.6	ns	0.4
Salt (%)	0.6	0.6	0.7	0.7	0.7	0.7	0.7	0.7	ns	0.0
WHC (%)	89.1^{a}	88.7^{a}	85.4^{b}	$87.5^{a,b}$	85.9^{b}	$87.9^{a,b}$	78.3^{d}	82.7^{c}	*	0.7
Cook loss (%)	5.7^{d}	5.9^{d}	$7.4^{b,c}$	$6.1^{c,d}$	$6.9^{c,d}$	$6.2^{c,d}$	9.7^{a}	8.3^{b}	*	0.3
TEF (%)	6.4^{e}	6.8^{e}	$10.7^{b,c}$	8.8^{d}	$9.8^{c,d}$	$9.7^{c,d}$	13.0^{a}	11.5^{b}	*	0.4
Fat Exudate (%)	6.5^{c}	$7.6^{b,c}$	$9.1^{a,b}$	$8.7^{a,b}$	$8.1^{a,b,c}$	$7.9^{a,b,c}$	9.2^{a}	$7.7^{a,b,c}$	*	0.2
T_{2b} (ms)	$3.8^{a,b}$	$3.1^{a,b}$	$3.5^{a,b}$	$3.2^{a,b}$	2.6^{b}	$3.4^{a,b}$	$2.9^{a,b}$	4.1^{a}	*	0.1
T_{21} (ms)	38.9	38.9	37.7	38.9	36.5	38.9	38.9	38.8	ns	0.2
T_{22} (ms)	$250.3^{a,b}$	$265.8^{a,b}$	234.0^{b}	$249.5^{a,b}$	$265.8^{a,b}$	$257.6^{a,b}$	283.6^{a}	241.4^{b}	*	3.9
P_{2b} (%)	3.5	3.4	3.4	3.4	3.2	3.3	2.4	3.5	ns	0.1
P_{21} (%)	86.9	90.0	87.9	90.0	88.8	90.7	90.9	90.0	ns	0.3
P_{22} (%)	9.7	6.6	8.8	6.6	8.0	6.1	6.6	6.5	ns	0.3

Where Control – 0.50% STPP only; Formulation 1- 0.20% STPP+0.22% AP+0.58% CSS; Formulation 2- 0.20% STPP+0.00% AP+ 0.80% CSS; Formulation 3- 0.06% STPP+0.94% AP+0.00% CSS

*-Significance levels at P<0.05 of the interaction between HPP treatment and formulations using 2-way ANOVA, ns- not significant

SEM – Standard error mean of the sample population

a-e –Mean values with different superscripts within a row are statistically different (P<0.05) from each other using Tukey's comparison

6.3.2 Effect of HPP on Emulsion stability

Emulsion stability results expressed as TEF (%) and fat exudate (%) are presented in Table 6-3. Results of 2-way ANOVA showed that the application of HPP to sausage formulations produced a desirable interactive effect on emulsion stability i.e. inclusion of HPP-treated meat produced a different effect on ($P<0.05$) different formulations. It was noted that HPP-treated meat produced larger impact, which reduced TEF (%) values for sausage formulations 1 and 3 (with AP), whereas in sausage formulation 2 (without AP), the value was insignificantly reduced when compared within the individual formulations. This result indicated that AP played a role in lowering TEF (%); however, no such effects were observed for fat exudate (%) values. A decreasing trend was observed in fat exudate (%) values for phosphate-reduced sausage formulations. HPP application reduced ($P<0.05$) fat exudate (%) values for formulation 3, whereas the reduction was insignificant in sausage formulations 1 and 2 when compared with their respective formulations with non-HPP treated meat. This reduction in TEF (%) and fat exudate (%) values indicate improved emulsion stability, which can be attributed to the structural changes produced in the myofibrillar proteins by HPP and which subsequently resulted in the increased binding of water and fat within the meat matrix (Furlán, Padilla, & Campderrós, 2014; Yang et al., 2021). Conversely, HPP produced the opposite trend in TEF (%) and fat exudate (%) values for control sausage formulations. The controls' TEF (%) and fat exudate (%) values increased when HPP-treated meat was used to formulate sausages; however, this increase was insignificant. This insignificance in the emulsion stability values of control formulations can be explained by the saturated highest level of emulsion stability produced by STPP (0.50%) in the control formulations.

Results of a one-way ANOVA comparing HPP-treated meat, phosphate-reduced sausage formulations with control formulations (traditional sausage) with non-HPP treated meat showed that TEF (%) values and fat exudate (%) values significantly differed among the formulations (Table 6-5). The TEF (%) and fat exudate (%) values analysis showed that reducing the phosphate concentration increased the TEF (%) and fat exudate (%) values. This concurs with O'Flynn et al. (2014), who showed that

emulsion stability decreased with decreased phosphate concentration. However, HPP treatment at 150 MPa for 5 mins improved the emulsion stability of phosphate-reduced sausages but could not match that achieved in the control formulation.

6.3.3 Effect of HPP on WHC

Results of sausage formulation WHC are presented in Table 6-3. It was evident from 2-way ANOVA results that HPP and sausage formulations had a significant interactive ($P<0.05$) effect on the WHC values. It was observed that the WHC values increased significantly for phosphate-reduced sausage formulations 3 with HPP-treated meat. There was an insignificant increase in WHC in formulations 1 and 2 when compared with their respective formulations with non-HPP treated meat; such effect was also observed in the control sausage formulation. This concurs with the decreased emulsion stability values discussed above for the same samples, where the control formulation had reached its saturated WHC level that could not be increased further. The increase in WHC of phosphate-reduced sausage formulations was due to the increased emulsion stability created by HPP treatment Studies by Grossi, Søltoft-Jensen, Knudsen, Christensen, and Orlien (2012) reported the increase in WHC by HPP treatment was due to the disruptions of electrostatic and hydrophobic interactions producing the increased myofibrillar solubilisation.

Results of one-way ANOVA (Control non-HPP treated vs HPP-treated formulations) showed that control performed equal to formulations 1 and 2. However, a significant difference was observed in HPP-treated formulation 3 compared to control values. This difference was due to the low phosphate concentration (0.06%) in formulation 3. As an overall discussion, HPP treatment increased the WHC of the phosphate-reduced sausage formulations to the level observed for control (traditional Sausage formulations) WHC values, except for formulation 3.

6.3.4 Effect of HPP on Cook loss

Cook loss values for sausage formulations are presented in Table 6-3. Data from 2-way ANOVA showed that, similar to WHC, HPP treatment and sausage formulations produced significant ($P<0.05$) interactive impact on cook loss values (specifically in formulations 1 and 3). HPP reduced cook loss values for all three phosphate-reduced sausage formulations, but it was only significant for formulation 3. For the control

formulation no significant modification was observed. This decrease in cook loss can be attributed to the improved emulsion stability of the sausage formulations. HPP application aided the meat protein depolymerisation and increased the solubility of myofibrillar proteins that formed a stable gel matrix, thereby reducing cook loss values (O'Flynn, Cruz-Romero, Troy, Mullen, & Kerry, 2014).

Similar to WHC, results of one-way ANOVA analysis of cook loss values has shown that sausage formulations 1 and 2 with HPP-treated meat had cook loss values similar to control sausage formulations, with non-HPP treated meat, containing 0.5% STPP. Also, the cook loss value for sausage formulation 3 was much higher ($P<0.05$) than the control. This was due to the low concentration of STPP in formulation 3 (0.06%). In general, HPP improved the cook loss values of phosphate-reduced sausage formulations.

6.3.5 Water mobility analysis using LF – NMR

LF-NMR technique is widely used to study water mobility and distribution within meat matrices (Han, Wang, Xu, & Zhou, 2014). There are three components in the distribution curve, and each component represents each water type in the meat matrix: bound water, myofibrillar/immobilised (or entrapped) water and free water. The relaxation time (T) and correlated water proportion percentage (P) of the three components, bound water (T_{2b}, P_{2b}); myofibrillar/immobilised water (T_{21}, P_{21}) and free water (T_{22}, P_{22}), were assessed to measure the water mobility within the meat matrix before cooking. The analysis of the interaction of the factors on the relaxation time distribution data showed that HPP treatment and sausage formulations produced a significant interaction effect on T_{2b} and T_{22} values; however, the values of this parameter were not significantly affected by the treatments. The only value affected was T_{22} in formulation 3. Similarly, In terms of population distribution, HPP did not produce any significant changes in the population (P_{2b}) of inner bound water of control or sausage formulations 1 and 2. The significant increase in P_{2b} values for formulation 3 was unexpected since the internal water is primarily unaffected by mechanical disturbance (McDonnell et al., 2013). HPP increased ($P<0.05$) P_{21} values for sausage formulations 1 and 2, whereas the increasing effect was insignificant in control formulations. Similarly, the application of HPP decreased ($P<0.05$) P_{22} values

for sausage formulations 1 and 2, whereas the decreasing effect was insignificant in control formulations. This decrease in values of P_{22} can be attributed to decreased cook losses and increased WHC since the free water in P_{22}, responsible for cook and drip loss, had migrated to the myofibrillar/immobilised population matrix P_{21}. Concerning formulation 3, HPP application was ineffectual in causing P_{21} or P_{22} values changes.

Results of one-way ANOVA and comparison of the population distribution values in Figure 6-1 and Table 6-5, showed that the phosphate-reduced sausage formulations with HPP-treated meat significantly differed from control formulations with non-HPP treated meat. All three phosphate-reduced sausage formulations had increased P_{21} (only significant for formulation 2) and decreased P_{22} values (significant for all formulations) compared with control formulations with non-HPP treated meat. The increase in P_{21} indicates the increase in myofibrillar/immobilised water, thus explaining the rise in WHC. Therefore, the application of HPP positively influenced the water mobility of sausage formulations.

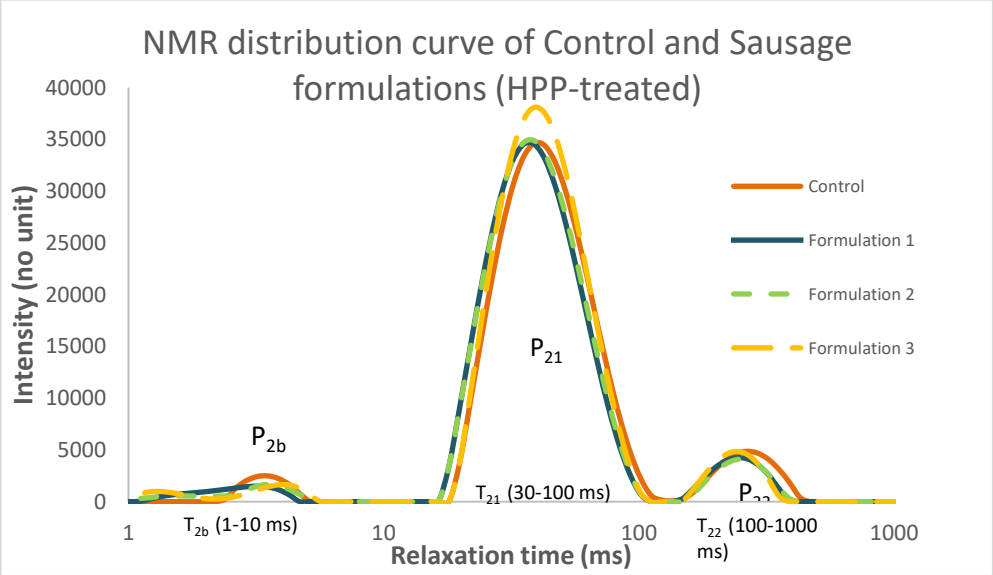


Figure 6-1: LF-NMR distribution curves of control formulations and HPP-treated phosphate-reduced sausage formulations.

Where Control – 0.50% STPP only; Formulation 1- 0.20% STPP+0.22% AP+0.58% CSS; Formulation 2- 0.20% STPP+0.00% AP+ 0.80% CSS; Formulation 3- 0.06% STPP+0.94% AP+0.00% CSS

6.3.6 Effect of HPP treatment on texture properties

The effect of HPP treatment on the hardness, chewiness, gumminess, springiness, and cohesiveness of breakfast sausages was measured and the results of Table 6-4 showed that the HPP and sausage formulations did not produce any significant interactive effect on these textural properties. The results observed that HPP treatment at 150 MPa for 5 min did not produce a significant impact on sausage hardness values for control and all three sausage formulations. This contrasts with the O'Flynn et al. (2014) study, which showed that HPP-treated meat (150 MPa for 5 min) improved hardness values for phosphate-reduced sausages (0.25%). Likewise, Yang et al. (2015) showed that HPP application improved sausage hardness values up to 200MPa. In our study, although non-significant, it was observed that the hardness values followed a reducing trend in the sausage formulations with HPP-treated meat, except sausage formulation 3. This decrease in sausage hardness can be attributed to the deterioration of functional proteins when treated with HPP (O'Flynn et al., 2014; Zhu, Yan, Yu, Wu, & Bennett, 2022). Similarly, the application of HPP in all sausage formulations produced a decreased effect in gumminess; however, these decreases were not significant, except for the treated control ($P<0.05$). A similar trend was observed for sausage chewiness and springiness values for all sausage formulations, where HPP effects were insignificant. This decrease in the textural values for sausage formulations is not desirable since it affects the quality by reducing product firmness.

The results of one-way ANOVA showed that HPP did not improve sausage textural properties, so reduced phosphate formulations cannot be enhanced under the research conditions employed in this study to equal the performance of control (0.5% STPP) sausage formulations non-HPP treated meat. It was observed that formulation 2 had the highest chewiness, gumminess and springiness values among formulations assessed, with formulation 3 having the lowest values for the same parameters. The cohesiveness values for all sausage formulations, including controls, were almost the same (0.70-0.80). However, the control formulations' hardness, chewiness, gumminess, and springiness values were higher ($P<0.05$) than all other sausage formulations with reduced phosphate and US-treated ingredients. This overall

observation shows why phosphates play a significant role in determining sausage texture characteristics. Although the literature review (See Chapter 1, Section 7.2.1, page 29) states that HPP improves the texture characteristics of meat products, the applied HPP treatment in this research to sausage formulations employed did not positively influence the texture properties of phosphate-reduced sausage formulations when compared to the control formulation.

6.3.7 Effect of HPP treatment on colour properties

The colour of meat products plays a significant role in determining consumer purchasing decisions. Thus, meat colour is an important quality attribute that requires attention when altering processed meat formulations (Tomasevic, Djekic, Font-i-Furnols, Terjung, & Lorenzo, 2021). Results of 2-way ANOVA showed that HPP treatment and sausage formulations did not form any significant interactive effect on colour parameters except for a* where a significant interactive effect was produced meaning that HPP treatment produced different effects on different formulations; more evident for formulation 3. It was observed from the results (Table 6-4) that the L* values for HPP treatment showed a very slight but insignificant increase in sausage formulations, including controls. A study by Zhu et al. (2022) showed that HPP application (100-400 MPa for 15 mins) increased L* values and decreased a* values in sausages. These changes in colour values were due to the denaturation of muscle proteins (Grossi, Søltoft-Jensen, Knudsen, Christensen, & Orlien, 2011). However, in contrast with this study, no a* value modification ($P>0.05$) was observed in sausage formulations following HPP treatment of meat employed in these formulations. Similarly, after HPP treatment, no significant changes were observed in b* values within the individual formulations.

Overall, from the results of one-way ANOVA, phosphate-reduced sausage formulations (1-3) containing HPP-treated meat had significantly lower L* values (60.63-61.68) compared with the control formulation (L*-65.32±1.76) containing 0.5% of STPP and non-HPP treated meat. This increase in the darkness of the sausage formulations can be attributed to the addition of AP and CSS to the sausage formulations. The phosphate-reduced sausage formulations observed no significant differences in a* or b* values. However, the results of perceptual colour difference

(ΔE) showed a clear colour difference (ΔE>2) in the phosphate-reduced sausage formulations when compared to the control (with non-HPP treated meat) since Mokrzycki & Tatol (2011) highlighted that a visible difference is observed for inexperienced observers if the colour difference (ΔE) is above 2. The observed colour difference was mainly due to the change in luminosity after US-treated AP and CSS inclusion in the phosphate-reduced sausage formulations. In contrast, HPP did not affect the sausages' colour properties.

6.3.8 Effect of HPP on Lipid Oxidation (TBARS value)

Lipid oxidation measures the oxidative degradation of lipids produced by the highly complex free radical reaction between fatty acids and oxygen, resulting in discolouration, drip losses, off-odour and off-flavour production, and the formation of potentially toxic compounds (Morrissey et al., 1998). This will result in the emergence of off-flavours and off-aromas in meat products as this value increases over time. In our previous chapter that analysed sausages from days 0, 3, 6 and 9, the TBARS values for all three phosphate-reduced sausage formulations containing US-treated AP and CSS were found to be similar to each other on day 3 (0.20 – 0.26mg MDA/kg) and day 6 (0.29-0.39mg MDA/kg) (See Chapter 5, Table 5-2, page 126). The main differences in TBARs were observed only on day 9, and hence, in this study, day 9 values were used to assess the effect of HPP on lipid oxidation.

Results of 2-way ANOVA showed that HPP treatment and sausage formulations produced significant interactive impact on day 9 TBARS values. In general, application of HPP increased TBARS values for most of the different pressure/time combination treatments, as previously reported (Omana, Plastow, & Betti, 2011). Studies have shown that HPP treatment applied between 300-600MPa induces lipid oxidation faster (Beltran, Pla, Yuste, & Mor-Mur, 2003; Cava, Higuero, & Ladero, 2021). However, from lipid oxidation values obtained on storage days 0 and 9 (Table 6-4), it was evident that HPP did not produce significant changes in TBARS values for sausage formulations 1 and 3, along with the control formulations when compared with their respective formulations with non-HPP treated meat. HPP treatment did not change TBARS values for either control or phosphate-reduced sausage formulations. Additionally, final TBARS values were within the detectable highest

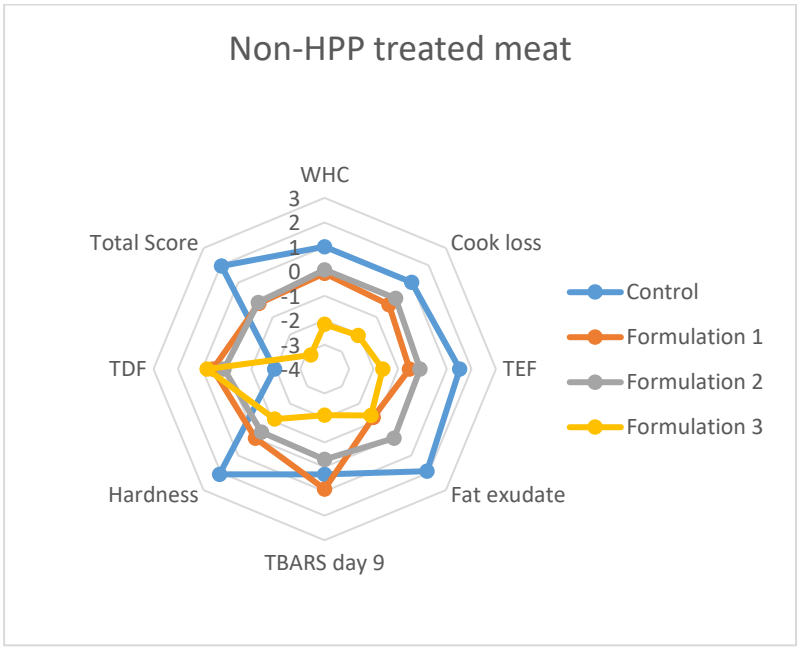
upper range threshold limit of 2.0-2.5mg MDA/kg, above which rancidity occurs with off-flavours and off-aromas and the formation of potentially dangerous free radicals (Zhang et al., 2019).

In addition, one-way ANOVA results showed TBARS values observed for control formulations (non-HPP treated meat) did not vary ($P>0.05$) with the three phosphate-reduced sausage formulations with HPP-treated meat. On further analysis, phosphate-reduced sausage formulation 3 (0.06% STPP) had a higher TBARS value, which might be due to its lower concentration of phosphate and lack of CSS since phosphates (Long et al., 2011) and CSS (Iriondo-DeHond et al., 2019) are excellent antioxidants. The values showed that HPP does not produce any induced oxidation, consequently playing no role in the quality degradation of phosphate-reduced sausage formulations.

6.3.9 Formulation grading

The formulation grading system indicated the overall performance of treatment on different formulations compared to whole sample populations. The total scores for formulations using Non-HPP treated meat were as follows, (i) Control: 1.96; (ii) Formulation 1: - 0.20; (iii) Formulation 2: - 0.16; (iv) Formulation 3: -3.19. Similarly, the total scores for formulations using HPP-treated meat were as follows, (i) Control: 1.41; (ii) Formulation 1: 0.17; (iii) Formulation 2: 0.87; (iv) Formulation 3: -1.08. It was evident that the control formulations possessed higher scores for non-HPP and HPP treatment, even after HPP treatment had reduced its overall score. It was also evident from Figure 6-2 that HPP treatment improved overall scores for phosphate-reduced sausage formulations to a greater extent. Sausage qualities, such as tenderness and lipid oxidation values, greatly influenced overall scores, besides those obtained for WHC and cook loss. It was also noted that STPP content influenced the scores. Although AP and CSS have almost similar fibre content, a difference in their STPP interaction was observed. Thus, HPP treatment in formulation 2, containing only CSS (0.80%), improved the overall score, making it the best phosphate-reduced sausage formulation for those studied. Conversely, formulation 3, containing only AP (0.94%), had the lowest total score. A more targeted grading system can be developed using the weighted arithmetic mean from a commercial perspective.

(i)



(ii)

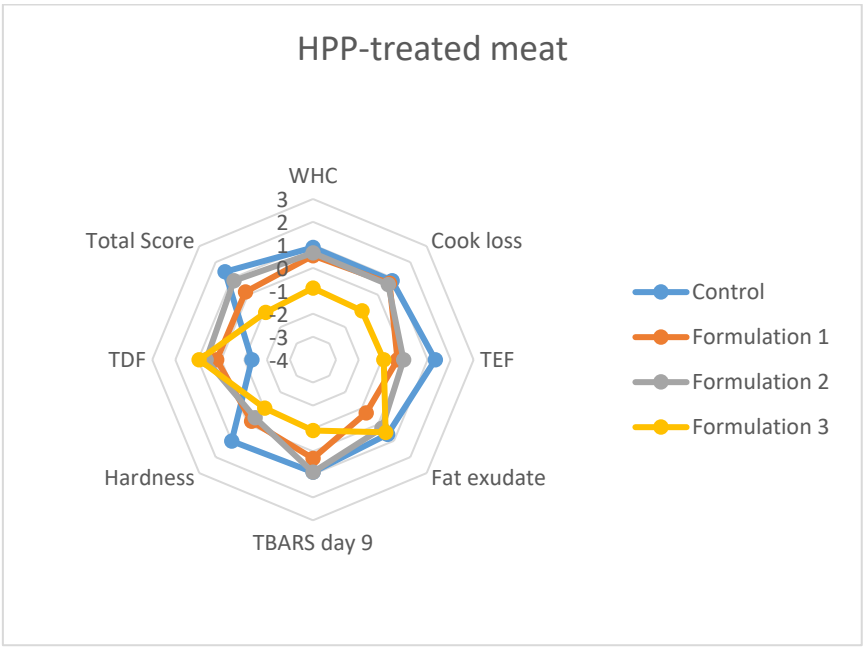


Figure 6-2: Scores for each one of the main physicochemical properties analysed are reported for control and sausage formulations 1-3. Sausage formulations with non-HPP treated meat; (ii) Sausage formulations with HPP-treated meat. The total score for each treatment (divided by 3) is presented.

Where Control – 0.50% STPP only; Formulation 1- 0.20% STPP+0.22% AP+0.58% CSS; Formulation 2- 0.20% STPP+0.00% AP+ 0.80% CSS; Formulation 3- 0.06% STPP+0.94% AP+0.00% CSS

Table 6-4: Mean and standard deviation values of colour, texture, and TBARS analysis of sausage formulations

		Control		Formulation 1		Formulation 2		Formulation 3		Interaction Sig.	SEM
		Non-HPP	HPP	Non-HPP	HPP	Non-HPP	HPP	Non-HPP	HPP		
L*		65.3	66.7	60.0	61.0	59.8	60.6	62.0	61.7	ns	
a*		$5.8^{a,b}$	5.7^{b}	$6.2^{a,b}$	$5.9^{a,b}$	$5.9^{a,b}$	$5.9^{a,b}$	$5.8^{a,b}$	6.3^{a}	*	0.1
b*		19.7	19.9	19.1	19.4	19.3	19.1	19.9	19.6	ns	0.1
Hardness (N)		48.2	39.3	30.9	29.1	28.0	27.3	21.7	22.4	ns	2.2
Chewiness (J)		109.9	81.3	31.4	25.9	38.4	35.1	15.2	14.7	ns	8.3
Cohesive force		0.7	0.7	0.7	0.7	0.7	0.7	0.8	0.8	ns	0.0
Gumminess (N)		13.3	10.4	5.0	4.1	6.0	5.2	3.2	3.1	ns	0.9
Springiness (mm)		8.2	7.6	6.4	5.7	6.3	6.5	4.7	4.7	ns	0.3
TBARS (mg MDA/kg)	**Day 0**	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.3	ns	0.0
	Day 9	$0.6^{b,c}$	0.4^{c}	$0.5^{b,c}$	$0.6^{b,c}$	0.7^{b}	$0.5^{b,c}$	1.0^{a}	0.8^{a}	*	0.0

Where Control – 0.50% STPP only; Formulation 1- 0.20% STPP+0.22% AP+0.58% CSS; Formulation 2- 0.20% STPP+0.00% AP+ 0.80% CSS; Formulation 3- 0.06% STPP+0.94% AP+0.00% CSS

*-Significance levels at P<0.05 of the interaction between HPP treatment and formulations using 2-way ANOVA, ns- not significant

SEM – Standard error mean of the sample population

a-e –Mean values with different superscripts within a row are statistically different (P<0.05) from each other using Tukey's comparison

Table 6-5: Mean values comparing the physicochemical properties Control (non-HPP treated meat) with phosphate-reduced sausages formulations with HPP-treated meat

		Non-HPP treated Control	HPP-treated formulations			Sig.	SEM
			Formulation 1	Formulation 2	Formulation 3		
WHC		89.1 ± 0.7^{a}	87.5 ± 0.4^{a}	87.9 ± 0.8^{a}	82.7 ± 1.7^{b}	*	0.7
Cook loss		5.7 ± 0.3^{a}	6.1 ± 0.2^{a}	6.2 ± 0.4^{a}	8.3 ± 0.5^{b}	*	0.3
TEF (%)		6.4 ± 0.4^{a}	8.8 ± 0.3^{b}	9.7 ± 0.1^{b}	11.5 ± 0.6^{c}	*	0.4
Fat exudate (%)		6.5 ± 0.4^{a}	8.7 ±0.9^{b}	7.9 ± 0.5^{ab}	7.7 ± 0.8^{ab}	*	0.2
P_{2b} (%)		3.5 ± 0.4	3.4 ± 0.1	3.3 ± 0.7	3.5 ± 0.0	ns	0.1
P_{21} (%)		86.9 ± 2.9^{a}	90.0 ± 0.2^{a}	90.7 ± 0.9^{b}	90.0 ± 0.3^{a}	*	0.3
P_{22} (%)		9.7 ± 2.5^{a}	6.6 ± 0.1^{b}	6.1 ± 0.4^{b}	6.5 ± 0.2^{b}	*	0.3
L*		65.3 ± 1.7^{a}	61.0 ± 0.8^{b}	60.6 ± 2.4^{b}	61.6 ± 0.9^{b}	*	0.4
a*		5.8 ± 0.4	5.9 ± 0.3	5.9 ± 0.6	6.3 ± 0.3	ns	0.1
b*		19.7 ± 0.7	19.4 ± 0.6	19.1 ± 0.5	19.6 ± 0.7	ns	0.1
ΔE			4.3	4.7	3.7		
Hardness (N)		48.2 ± 1.6^{a}	29.1 ± 1.9^{b}	27.3 ± 6.6^{b}	22.4 ± 0.7^{b}	*	2.2
Chewiness (J)		109.9 ± 13.5^{a}	25.9 ± 0.3^{b}	35.1 ± 20.9^{b}	14.7 ± 3.0^{b}	*	8.4
Cohesive force		0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.8 ± 0.0	ns	0.0
Gumminess (N)		13.3 ± 0.5^{a}	4.1 ± 0.2^{b}	5.2 ± 2.4^{b}	3.1 ± 0.3^{b}	*	0.9
Springiness (mm)		8.2 ± 0.7^{a}	5.7 ± 0.1^{bc}	6.5 ± 1.0^{abc}	4.7 ± 0.4^{c}	*	0.3
TDF (%)		0.8 ± 0.1^{a}	3.6 ± 0.2^{b}	4.2 ± 0.2^{b}	4.6 ± 0.8^{b}	*	0.4
Ash (%)		2.1 ± 0.0^{a}	1.9 ± 0.1^{ab}	1.9 ± 0.1^{ab}	1.8 ± 0.1^{b}	*	0.0
Salt (%)		0.6 ± 0.0	0.7 ± 0.1	0.7 ± 0.1	0.7 ± .1	ns	0.0
TBARS (mg MDA/kg)	Day 0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	ns	0.0
	Day 9	0.6 ± 0.1	0.6 ± 0.0	0.5 ± 0.0	0.8 ± 0.0	ns	0.0

Where Control – 0.50% STPP only; Formulation 1- 0.20% STPP+0.22% AP+0.58% CSS; Formulation 2- 0.20% STPP+0.00% AP+ 0.80% CSS; Formulation 3- 0.06% STPP+0.94% AP+0.00% CSS

*-Significance levels at P<0.05 between formulations with HPP-treated meat and control formulations with non-HPP treated meat using one-way ANOVA, ns- not significant

SEM – Standard error mean of the sample population

a-c –Mean values with different superscripts within a row are statistically different (P<0.05) from each other using Tukey's comparisons.

6.4 Conclusion

The present study revealed that HPP treatment positively affected most qualitative properties for phosphate-reduced breakfast sausage formulations containing US-treated AP and CSS. In addition, HPP treatment produced significant interaction effect with sausage formulations on important properties like WHC, cook loss and emulsion stability, thus proving that different formulations reacted differently with the addition of HPP-treated meat. This interactive effect seems to be related to the AP content in each of the formulations. This explains the improvement in WHC, cook loss, and emulsion stability values for these novel sausage formulations when HPP was applied at 150MPa for 5 min. The lower values achieved for textural parameters could prove helpful in formulating processed meat products for the aged population, who have difficulty chewing meat. Further analysis of the results obtained in this study showed that sausage formulation 2, containing 0.20% STPP (60% phosphate reduction) and 0.80% CSS, produced the best quality results when compared to the other experimental formulations, proving that CSS has much better scope for replacing the phosphates in sausages than AP. In conclusion, HPP and inclusion of US-treated AP and CSS in phosphate-reduced sausage formulations could successfully be used in commercial Irish breakfast sausage manufacture without any perceived loss in sensory attributes or other quality parameters.

Chapter 7. General Discussion and Conclusions

7.1 General Discussion

For centuries, meat and meat products have been widely consumed as an integral food source around the globe (Moreira et al., 2022) since they are an excellent source of proteins, fatty acids, essential amino acids, minerals, and vitamins (Illippangama, Jayasena, Jo, & Mudannayake, 2022). The increase in global population and a rise in personal income have increased global meat consumption by 58% over 20 years to 2018, reaching a global consumption of 360 million tonnes of meat per year (Whitnall & Pitts, 2019). With the current level of per capita consumption, it has been forecasted that the global demand for meat and meat products will peak at 2.0 billion tonnes by 2050, given the population and the consumption rate remains the same (Henchion et al., 2021). However, along with this increasing global demand, there has been a recent growth in consumers' awareness related to the health concerns over the consumption of meat, especially processed meat products, which includes the products prepared using processing techniques such as salting, curing, marinating, emulsifying, smoking, or drying techniques. These perceived consumer concerns have been related to the higher usage of salts, fats, and other additives in processed meat products, like nitrate, nitrite or phosphates. In addition, there has been an increasing consumer demand for healthier and higher-quality meat products, followed by another growing global consumer trend, the 'clean-label' initiative, which encourages the minimal usage of artificial, non-organic additive ingredients in food products (Asioli et al., 2017). To address these issues, the collective meat industry has implemented various changes and innovations in product formulations and processing techniques. One such essential change in meat product formulation has been the removal or reduction of ingredients with negative consumer perceptions, for example, it is more frequent to find in the market diverse claims such as 'reduced-salt', 'fat-free', 'reduced-fat', 'natural', 'clean-label', and 'nitrate-free' in processed meat products. These processed meat products are produced by replacing negatively perceived and synthetic additive ingredients with alternative natural ingredients without negatively affecting the products' eating and technical characteristics.

Phosphates are one such synthetic additive widely used in processed meat products (ham, sausages, burgers, canned fish and so on) for their capability in improving water holding capacity (WHC), emulsion stability, stabilising pH, decreasing cook loss and enhancing sensory properties (Long, Gál, & Buňka, 2011). Importantly, they work synergistically with salt (NaCl), thereby reducing the salt concentration in processed meat products (Moiseev & Cornforth, 1997). The literature review (Chapter 1) of this thesis explored the various functionalities of different types of phosphates and their importance in maintaining the quality of processed meat products. Additionally, the literature pertaining to the impact of phosphate intake on consumer health was also reviewed. It has been reported that the maximum permitted usage of phosphates in processed meat products is 5000 mg/kg expressed as P_2O_5 content (Regulation (EC) 1333/2008, 2008) and the acceptable daily intake of Phosphorus (P) for a healthy adult is 40 mg/kg body weight per day (Younes et al., 2019). The increased consumption of processed meat products contributes to the increased intake of P, which was reported to increase elevated blood phosphate levels in patients with chronic kidney disorders (CKD) and also causes bone degradation in healthy individuals by inhibiting calcium absorption (Pinton et al., 2021). In a study by Byrne et al. (2021), on the implications of phosphate additives on people with chronic kidney disorders (CKD), about 72% of packaged meat and poultry in five leading supermarket chains in Ireland contained phosphate additives as part of their formulation. In 2010, the EU Commission on Regulation (EC) No 257/2010 recommended the European Food Safety Authority (EFSA) to re-evaluate the use of phosphate additives with high priority (Byrne et al., 2021). This, along with the global consumer trends discussed above, has resulted in the development of interest in removing phosphate additives from processed meat products, which was the main focus of this thesis research. However, the complete elimination of phosphates from processed meat products poses significant challenges, since its removal negatively affects the technical and quality attributes of the developed product.

The consumer acceptance of the phosphate-removed processed meat products mainly depends on the products' ability to replicate the sensory and eating characteristics of the traditional products. Thus, the literature review (Chapter 1)

evaluated the primary replacing techniques used by industries to address these consumers' demands, in order to remove or replace ingredients from processed meat products without affecting the products' quality. The two essential strategies discussed were (i) natural-alternative ingredient strategy and (ii) novel processing technology strategy.

The alternative ingredient strategy section of the literature review explored the potential natural ingredients (starch, proteins, and fibres) that could mimic phosphates' functionality and assessed their ability to replace the phosphates in processed meat products. Among the various discussed ingredients, dietary fibres were excellent alternative ingredients based on the criteria explained in the following sentences, which could improve the quality of meat products in terms of technological, nutritional and health aspects (Santos et al., 2020). Adding dietary fibres to meat products could improve the WHC, OHC, emulsion stability and other quality characteristics (Ahmad, Khalid, & Younis, 2020). In addition, dietary fibres would enhance the natural fibre content of the meat products, which are naturally lacking in this component, along with the health benefits associated with their intake, for example, inhibiting the incidence of colon cancers (Ahmad et al., 2020). After reviewing the potential benefits of dietary fibres as phosphate replacers, choosing the adequate source of this functional ingredient was also considered in a further step. In this sense, and trying to promote a circular economy and a more sustainable food industry, a particular interest in the revalorisation of food co-products ingredients rich in dietary fibres was developed throughout the Ph.D. study. Based on the evidences from scientific researches (Martuscelli, Esposito, & Mastrocola, 2021; Rather et al., 2015) and preliminary physicochemical properties analyses, two food processing co-products, apple pomace (AP) and coffee silver skin (CSS), rich in dietary fibres, were chosen as the ingredients of interest in this thesis research. Chapter 1 reviewed the techno-functional benefits of these two ingredients in processed meat products in detail. The high dietary fibre content (~ 86% of TDF for both AP and CSS) and the low natural phosphorus content (AP – 1.4 g/kg; CSS – 1.5 g/kg) of these ingredients, along with their functionality, were the significant factors

when selecting them as the potential phosphate replacers for experimental work in this thesis research (Chapter 3-6).

Finally, followed by the ingredient strategy, the technological strategy section of the review explored the various novel processing technologies, with the potential to be widely used by the food industries, to improve the quality of food products with reduced or replaced ingredients (e.g. salt-reduced, low-fat, phosphate-free foods). As a result, among the various novel food processing technologies revised, the two most interesting emerging processing technologies, within the scope, were determined to be explored as part of the experimental work in replacing the phosphate content in meat products. They were: (i) power ultrasound (US) (explored in Chapters 4 and 5) and (ii) high-pressure processing (HPP) (explored in Chapter 6). The selection of both technologies to examine their phosphate-replacing ability was based on the following criteria: their ability to modify the physicochemical properties of ingredients and promote the natural solubilisation of meat proteins (required for good textural and technical traits related to water and oil holding capacity). Such technologies are widely used in multiple scientific research studies to improve the quality of meat and meat-containing products, especially the ingredients used in processed meat products. The review extensively covered the advantages and disadvantages of these technologies in developing healthier processed meat products (See Chapter 1, Section 7, pages 25-30) and how they could be applied specifically in processed meat products with reduced-phosphate content. While HPP has been a well-established novel processing technology with wide industrial applications on meat products, the industrial application of US technology on meat products is still underdeveloped, covering only up to laboratory research scale since there is a lack of standardised parameters and methodologies.

One of the main conclusions from the detailed literature review was that when phosphate was removed entirely from meat products, their quality lowered drastically despite the individual application of the two above-discussed strategies. However, few such changes were observed in the reviewed reports whose design focused on reduction rather than removal. Thus, considering what has been discussed in the previous paragraphs, a detailed thesis study on phosphate reduction

was conducted by following both strategies individually and, as a more globally-novel approach, exploring the synergies between novel ingredients, and emerging processing technologies, to develop a phosphate-reduced final product with good quality and increased fibre content. From a national perspective, Irish breakfast sausages are one of the widely consumed processed meat products that depend on phosphates to maintain their techno-functional and sensory attributes; hence, this combined approach was used to develop good-quality phosphate-reduced Irish breakfast sausages in the following experimental chapters (Chapters 3-6).

Consumers are the primary source of demand for all food products, and they drive the significant developments carried out by the food industries to fulfil their requirements. Their acknowledgement of the above-discussed phosphate-reduction strategy and their acceptability of phosphate-reduced processed meat products are essential for adopting and commercialising these products by the meat industry. Henceforth, parallel to the experimental studies involving the strategical approach, a survey was conducted to measure the Irish consumers' (n=548, female 322; male 226) demand for phosphate-reduced processed meat products and their actual intention to purchase them (Chapter 2). This study used the extended Theory of Planned Behaviour (TPB) method to study the consumers' purchase intention since TPB has been extensively used to study people's social behaviours (Ajzen, 1991), using the model construct variables attitudes, social norms, perceived behavioural control (PBC), perceived health risks and intention. The analysis of consumers' knowledge of phosphate additives and their harmful effects showed that their level of understanding of the functionalities of phosphates was low compared with that of other additives, especially salt (Fellendorf, Kerry, & O'Sullivan, 2018). Furthermore, the construct analyses of the extended TPB model used in this study showed that the consumers' purchase intention towards phosphate-reduced processed meat products was positively influenced by the attitude, subjective norms and perceived health risks constructs. Unfortunately, the effect of PBC that measured the ease of purchase by the consumer was non-significant in this study, implying that consumers believed that they did not have enough facilities to purchase these products. However, despite this insignificance and the lack of proper knowledge, about 70% of

the total survey population emphasised that reducing phosphate additives in processed meat products is essential, which was explained by consumer demands to remove any synthetic additives used in processed meat products, as discussed previously.

As the main conclusion from Chapter 2, Irish consumers would be willing to purchase phosphate-reduced processed meat products of a different quality than original products, indicating the importance and future scope of this current thesis research. Furthermore, to improve this consumer's purchase intention and to convert this purchase intention into behavioural action, various recommendations based on significant variables were discussed in the study from the perspective of policymakers, scientific researchers, and the meat industry (See Chapter 2, Section, 2.5.1, page 55). This included the implementation of product reformulation and technology strategies. Interestingly, results of meat consumption frequency from survey work indicated that sausages were the most regularly consumed (95% of consumers) processed meat products among the other listed products, thus, supporting our decision to work on product reformulation of Irish breakfast sausages in the further thesis chapters. This approach is considered as highly novel since this is the first worldwide study conducted to understand the consumer behaviour towards the phosphate-reduction and phosphate-reduced processed meat products using the consumer social behaviour theory. In addition, this study could be used as a reference for futuristic research studies involving the additive reduction strategies in various other types of processed meat products.

Following the consumers' interest in phosphate reduction in processed meat products (from Chapter 2), an experimental study (Chapter 3) on phosphate reduction in Irish breakfast sausages was conducted using the natural alternative ingredients strategy involving AP and CSS, as discussed in the literature review. This chapter assessed the phosphate-reducing ability of AP and CSS. To validate this hypothesis, 18 different sausage formulations with varying levels of sodium tripolyphosphate (STPP) (0-0.5% w/w) and natural fibre-rich ingredients (AP and CSS; 0-1.0% w/w) were developed using an experimental mixture design approach and were evaluated regarding their physicochemical properties. The primary reason for

using this mixture design approach was that it required lesser experiments to examine all the factors' effects, thereby providing the optimum combination of all subjected variables (Aydar, 2018). The combined use of both ingredients (AP and CSS) and mixture design approach improved the originality of the study and reduced the issues associated with the determination of optimum ingredient mixtures in reducing the phosphate concentration.

Results indicated that the most significant physicochemical properties for meat products, such as WHC, cook loss and textural properties, of the sausages degraded with reduced STPP concentration. These findings were in accordance with those of Glorieux et al. (2017), which showed the quality of emulsified meat products decreased with reduction in phosphate concentration. Results also showed that the sausage formulations with STPP entirely replaced (0% w/w STPP) by either of the ingredients or the combination of both ingredients recorded the poorest quality attribute values, with decreased WHC, increased cook loss and decreased textural properties compared with other formulations with reduced phosphate (0.2-0.4% w/w of STPP) concentrations. This supports the fact that phosphates play a significant role in maintaining the quality of processed meat products (especially the emulsified products), and it is a complex technological challenge to eliminate them without compromising product quality. Thus, these results support our approach of reducing the phosphate content instead of focusing on removing them completely, as previously discussed. It was also noted that the addition of AP and CSS in sausage formulations with reduced STPP (0.2-0.3% w/w) recorded WHC and cook loss values close in range to that of sausages with higher STPP (0.5%). This implies that the dietary fibre content of AP and CSS played a role in improving the WHC and cook loss values in these products.

Once the obtained results were analysed, these were used as model responses in the mixture design software for optimisation. Since the focus of the research was to reduce STPP content, constraints were set to provide optimum concentrations with lower STPP (0-0.2%) and higher AP and/or CSS (0-1%) content in addition to conditions required to obtain desirable quality parameter values (See Chapter 3, Section 3.7, page 82). The desirability method in the mixture design software

predicted three optimised phosphate-reduced sausage formulations (See Chapter 3, Section 3.7, page 82). As a general outcome, the optimised formulations considerably reduced STPP addition from 0.5% w/w to the range of 0.06-0.20% w/w, with acceptable desirability values (45-67%) for techno functional properties. At the same time, it successfully demonstrated AP and CSS's phosphate-reducing ability. Additionally, this study showed the novel possibility of enriching sausages with dietary fibres (2-5% w/w) that could serve consumer health benefits with minimal loss in product quality.

Although the ingredient strategy approach reduced phosphate concentration in sausage formulations up to 80% with better desirability (65-67%), predicted physicochemical properties values were lesser than retail control (0.5% STPP) sausages (Chapter 3); implying that consumers may not accept this product and that there is still room for further formulation improvement. Hence, different possibilities to improve sausage quality parameter values were explored, and the second strategy for phosphate-reduction (i.e., application of novel processing technology to enhance the characteristics of the ingredients), was suggested as one potential solution. As previously outlined, US can be used to improve the techno-functional properties of food ingredients by physically modifying those (Inguglia et al., 2018). Therefore, it was hypothesised that US could be applied to modify the structure and potentially enhance the techno-functional properties of AP and CSS when treated individually; and thus, further strengthening the ingredient's ability to replace the phosphates without affecting the physicochemical properties of the meat products in which they were imparted.

Chapter 4 studied the effect of US (250 W, 20 kHz, US probe system) at different application time (15 and 30 mins) on ingredients dispersions (10% w/v). Characterisation results of US-treated AP and CSS were compared with that of control (untreated) and freeze-dried ingredients (to eliminate the effect of freeze-drying) to analyse the overall impact of US on AP and CSS. Results showed that no significant changes were produced by applying US on the proximate composition and total dietary fibre (TDF) content values of both ingredients, except for moisture values, which decreased significantly for both AP and CSS due to freeze-drying and

US-assisted drying kinetics (Zhu et al., 2021). On the other hand, the application of US significantly improved the water absorption capacity (WAC) and oil absorption capacity (OAC) of AP; however, in the case of CSS, there was an insignificant increase in WAC and OAC values. This insignificance for CSS was explained by the close range of particle size of CSS since the values of WAC and OAC are highly influenced by the particle size of the ingredients (Ballesteros, Teixeira, & Mussatto, 2014). However, for both AP and CSS, WAC and OAC values had increasing trends as treatment durations increased. Particle size distribution results showed that the surface mean diameter D (3, 2) significantly increased for both AP and CSS with an increase in treatment duration. The increase in WAC and OAC values was explained by the exposure of internally hidden hydrophilic and hydrophobic groups caused by the application of US, which may disrupt the native structure of fibres. Furthermore, colour difference (ΔE) analysis showed that both US-treated AP and CSS presented visible colour differences compared to their respective controls due to the cooking effect of US. The impact of this colour change was taken up in the next thesis section (Chapter 5).

Considering the ingredients' properties associated with the technical traits of breakfast sausages (WAC, OAC and particle size), this study reaffirmed the role of US technology in improving the techno-functional properties of the ingredients. Moreover, the different analyses conducted in this study concluded that the ingredients treated for a longer duration (30 mins) of US produced better results than those treated for 15 mins. The results of this study also provided the prospects of future studies involving the application of US at different frequency, different mode of application (ultrasonic water bath) and different range of ingredients (meat and other meat associated ingredients). The positive impact observed on the ingredients' performance when US was applied can be advantageous when developing new formulations, and this potential was further investigated in the following experimental work (Chapters 5 and 6).

Thus, Chapter 5 combined ingredient strategy (Chapter 3) with technological strategy (Chapter 4) by introducing the techno-functionally improved, US-treated (30 mins) AP and CSS in the three optimised phosphate-reduced sausage formulations

(obtained from Chapter 3). This was designed in order to assess whether the improvement observed in the functionality of the ingredients after treatment will also have an impact when incorporated into a real food matrix. Therefore, improvement in the physicochemical properties of sausages, produced by the US-treated ingredients, was investigated and compared with the sausage formulations containing non-US treated AP and CSS (See Chapter 5, Section 2.2, page 113). Values obtained for the formulations with non-US treated AP and CSS in this chapter were very close to software predicted values (Chapter 3), indicating consistency of both production process and ingredient performance. Results showed that application of US-treated AP and CSS to phosphate-reduced sausage formulations did not produce any significant interactive changes in the proximate composition, since US-treatment did not produce any notable changes in the proximate content of ingredients. As hypothesised in the previous paragraph, application of US-treated AP and CSS with improved WAC produced significant interactive impact on sausage formulations and significantly improved the WHC of all three sausage formulations compared to their equivalents. This, in turn, significantly decreased the cook loss values, since cook loss and WHC were inversely correlated to each other. Similarly, the improved OAC values of treated AP and CSS resulted in desirable decreasing value trends of total expressible fluid (TEF %) and fat exudative (%) loss, thus enhancing emulsion stability of sausage formulations. However, this decrease in emulsion stability was insignificant in terms of fat exudate (%) values to prove the interactive effect of incorporating US-treated AP and CSS in contrast to non-US treated equivalents. Although US-treatment produced visible colour difference (ΔE) in the ingredients (Chapter 4), no colour differences were observed in their inclusion, owing to their lower concentration in sausage formulations. Regarding textural properties, addition of US-treated AP and CSS did not produce any changes in product hardness; however, a reducing trend was observed for springiness, chewiness, and gumminess values for all sausage formulations. The ingredients also negatively affected the quality of sausage formulations, in terms of lipid oxidation, with higher day 9 TBARS values (but still under the threshold limit) compared to control sausage formulations. In conclusion, combining alternative ingredient and technology (US) strategies by adding US-treated AP and CSS to the sausage formulations improved their specific

physicochemical properties such as WHC, cook loss and emulsion stability, while keeping the phosphates level at 0.06-0.20% w/w. Regarding Texture Profile Analysis, new product formulations produced softer textural properties and undesirable lipid oxidation values. Another major challenge of this study was the uncertainty around the consumption of US-treated ingredients due to the lack of official regulations by a recognised food body (e.g. Food Safety Authority Ireland-FSAI; European Union Food Commission-EFSA) and safety concerns. This, in turn, has resulted in our decision to avoid conducting sensory analysis of the products, as discussed in the literature review and thesis objective (See Chapter 1, Section 1.7.3.1 & 1.8, pages 31 & 33). However, there are potential chances of its food application in the near future owing to their various applications in food and biochemical industries (Priyadarshini et al., 2019).

In the previous Chapter 4, the technological approach was focused on treating functional ingredients; however, in Chapter 6, it was hypothesised that this same approach could be applied to the meat itself in order to improve its functionality and overcome the loss of quality observed when phosphate was reduced in sausage formulations. In this sense, the application of a new technological approach, HPP, was assessed since it has been reported that this technology can modify the functionality of meat proteins and impact the final product's technological traits (Andreou et al., 2017). Therefore, in this study, pork meat was treated with HPP (150 MPa, 5 mins) before its addition to the sausage batter. As per Flynn et al. (2014), the selected HPP parameters, employed in this work were reported to have improved the textural properties of phosphate-removed sausages. Thus, each phosphate-reduced sausage formulation with US-treated ingredients was prepared using the HPP-treated meat and compared to their respective formulations made from non-HPP treated meat (See Table 6-1, page 136). It also included US-treated ingredients to understand the impact of HPP on final product quality.

The inclusion of HPP-treated meat did not produce any significant changes in the proximate composition of sausage formulations. A significant interactive increasing trend (i.e. different formulations were differently affected by the HPP treatment) was observed in emulsion stability values (TEF (%) & Fat exudate (%)) as a result of

treating the pork meat with HPP. Results of WHC and cook loss showed significant and positive interaction between HPP treatment and the three formulations tested, with usage of HPP-treated meats, thereby confirming the increased WHC and decreased cook loss values obtained by Flynn et al. (2014). However, textural parameters presented showed insignificant decreasing values, which may not be desirable for normal adults, but would be more acceptable to an elderly or infirm adult population with chewing and swallowing (dysphagia) difficulties. Interestingly, the addition of HPP-treated meat insignificantly decreased the lipid oxidation values of sausage formulations. This contradicted previous reports on HPP-treated meat products since HPP was reported to induce lipid oxidations in meat products (Cava, Higuero, & Ladero, 2021). Thus, as an overall implication, the highly novel approach of combining the application of alternative ingredients and novel technology (US and HPP) approaches along with alternative ingredients (AP and CSS) have been used to develop phosphate-reduced Irish breakfast sausages without any or reduced adverse change in their physicochemical properties, and that could be adopted by consumers. This could be further used to produce the wide range of phosphate-reduced processed meat products with improved quality.

The experimental design employed in Chapters 5 and 6 did not allow for calculating a desirability score, as in Chapter 3. Hence, a scoring system was used to identify the best optimum formulations. In this scoring system, all the parameters evaluated, including TDF and functionality, were standardised and added up into a final score (more details are presented in Chapters 5 and 6). Particularly in Chapter 5, the comparison was between sausage formulations, including US-treated and non-US treated functional ingredients (See Figure 5-3, page 129); while in Chapter 6, the comparison was between sausage formulations (with HPP and non-HPP treated meat) along with the control (traditional) Irish breakfast sausage (STPP-0.5%) without any novel processing treatment and natural ingredients (AP and CSS) (See Figure 6-2, page 151). It should be noted that in both chapters (5 and 6), the three formulations employed were those optimised in Chapter 3 (See Chapter 3, Section 3.7, page 82). Based on the developed scoring system and from the results of Chapter 5, it was concluded that STPP had a considerable influence on the quality of sausage

formulations since formulation 3 (STPP-0.06%) had lower scoring values in all analyses than formulations 1 (STPP-0.2%) and 2 (STPP-0.2%). Similarly, formulation 3 in Chapter 6 recorded the lowest values for all studies compared to 1 and 2. The main improvement observed in Chapter 6 was that the sausage formulations 1, 2 and 3 had higher TDF values than the control. Additionally, WHC values improved throughout the sequence of chapters, and WHC values for formulations 1 and 2 were statistically similar in the range of retail control (0.5% STPP) sausages studied in Chapter 6. Scoring system results in Chapter 5 showed that formulation 2 had the highest scores compared to formulations 1 and 3. Similar to Chapter 5, Chapter 6 indicated that formulation 2 had the highest scores compared to other formulations (1 and 3). However, the score for formulation 2 does not match the control, further confirming our above statement at the beginning of the discussion, i.e. removal/reduction of STPP concentration negatively influences the quality of sausages.

In conclusion, formulation 2, primarily consisting of CSS, yielded the best overall results for the parameters evaluated during the performance of this research work; therefore, it can be considered that CSS is the ingredient with the most potential as a phosphate replacer, which positively interacts with the US and HPP treatment, thereby improving the quality of sausages. Focusing more on the technological parameters (US and HPP), a further improvement in the physicochemical properties of sausages could be achieved than the one obtained just by the novel ingredients approach, implying that both approaches should be considered holistically when designing new formulations and processes for phosphate-reduced Irish breakfast sausages. These techniques and technologies could potentially be employed to reduce or remove the phosphate content from various processed meat products with a broader focus.

7.2 Conclusions

For decades, meat researchers have been examining the various innovative scientific possibilities that could be adopted and commercialised by the meat industries to produce high-quality and healthier meat products and satisfy the consumers' demand. Among the various approaches explored, this thesis investigated two major strategies namely, (i) alternative natural ingredient strategy using two dietary fibre-rich ingredients, AP and CSS and (ii) novel processing technology strategy using US and HPP, to produce phosphate-reduced Irish breakfast sausages without any changes in the physicochemical properties. The advantages of using natural alternative ingredients are that they can be used to produce 'natural', 'free-from' meat products by replacing the synthetic ingredients and also allows the producers to fortify the products with characteristics that are not naturally present. In our case, the meat products are added with dietary fibre content by the addition of US and CSS. Similarly, application of novel processing technologies has advantages such as the ability of US to modify the functional properties of ingredients and the ability of HPP to alter the structure of meat proteins. They also have improved efficiency and could reduce the processing duration compared to the traditional processing techniques. Based on the result of this thesis, the individual application of the alternative natural ingredient strategy was able to produce phosphate-reduced sausage formulations (0.06-0.2% STPP) with reduced physicochemical properties compared to the retail sausage with a higher STPP concentration. However, these ingredients along with novel processing technologies improved the various physicochemical properties of sausage formulations. It was also concluded that CSS had higher phosphate replacing ability compared to AP. In conclusion, with more focus towards the technology (US and HPP) parameters, further quality improvement in the sausages could be obtained. In addition, with further experimentation, these approaches could to potentially used to produce various other phosphate-reduced or –replaced processed meat products. The consumer survey study in this thesis also revealed that consumers showed positive intention to purchase these products if they are widely available. Hence the novelty of this thesis work could be used as an evidence to produce such healthier meat products in industrial scale.

7.3 Future Investigation

With the results obtained from this thesis research as a foundation, the various potential future research works that could improve the quality of processed meat products are discussed below.

- Future research should focus on reducing the phosphate contents in other processed meat products such as burgers and hams, emphasising the injected meat products using natural co-product ingredients and novel processing technology.
- Further studies on physicochemical improvement of co-products ingredients AP and CSS should be made with the variable frequency levels of US treatment using high power non-contact ultrasound transducers or ultrasonic baths.
- A separate study should focus on optimising US treatment of raw meat to improve their quality.
- A study should be made on the US or HPP treatment of the entire product with improved natural alternate co-product ingredients.
- To validate the industrial application of this current thesis research, future studies must focus on the sensory acceptability of the products.
- A separate study can be made by increasing the fibre content added to the products
- A specially focussed study on salt reduction can be made combining both the ingredient and technology strategy.
- In addition, special attention should be given to the cost and energy consumption of these technologies, such as HPP and US.

References

- Ağar, B., Gençcelep, H., Saricaoğlu, F. T., & Turhan, S. (2016). Effect of sugar beet fiber concentrations on rheological properties of meat emulsions and their correlation with texture profile analysis. *Food and bioproducts processing, 100*, 118-131. doi: 10.1016/j.fbp.2016.06.015
- Ahmad, S. S., Khalid, M., & Younis, K. (2020). Interaction study of dietary fibers (pectin and cellulose) with meat proteins using bioinformatics analysis: An In-Silico study. *Food science & technology, 119*, 108889. doi: 10.1016/j.lwt.2019.108889
- Aitken, R., Watkins, L., Williams, J., & Kean, A. (2020). The positive role of labelling on consumers' perceived behavioural control and intention to purchase organic food. *Journal of cleaner production, 255*, 120334. doi: 10.1016/j.jclepro.2020.120334
- Ajzen, I. (1985). From intentions to actions: A theory of planned behavior *Action control* (pp. 11-39): Springer.
- Ajzen, I. (1991). The theory of planned behavior. *Organizational behavior and human decision processes, 50*(2), 179-211.
- Ajzen, I. (2011). The theory of planned behaviour: Reactions and reflections (Vol. 26, pp. 1113-1127): Taylor & Francis.
- Alarcon-Rojo, A. D., Carrillo-Lopez, L. M., Reyes-Villagrana, R., Huerta-Jiménez, M., & Garcia-Galicia, I. A. (2019). Ultrasound and meat quality: A review. *Ultrasonics sonochemistry, 55*, 369-382. doi: 10.1016/j.ultsonch.2018.09.016
- Alarcon-Rojo, A. D., Janacua, H., Rodriguez, J. C., Paniwnyk, L., & Mason, T. J. (2015). Power ultrasound in meat processing. *Meat Science, 107*, 86-93. doi: 10.1016/j.meatsci.2015.04.015
- Al-Swidi, A., Huque, S. M. R., Hafeez, M. H., & Shariff, M. N. M. (2014). The role of subjective norms in theory of planned behavior in the context of organic food consumption. *British Food Journal*.
- Álvarez, C., Drummond, L., & Mullen, A. M. (2018). Protein recovered from meat co-products and processing streams as pork meat replacers in Irish breakfast sausages formulations. *Food science & technology, 96*, 679-685. doi: 10.1016/j.lwt.2018.06.020
- Álvarez, S., Mullen, A. M., Hamill, R., O'Neill, E., & Álvarez, C. (2021). Dry-aging of beef as a tool to improve meat quality. Impact of processing conditions on the technical and organoleptic meat properties *Advances in Food and Nutrition Research* (Vol. 95, pp. 97-130): Elsevier.
- Amiri, A., Sharifian, P., & Soltanizadeh, N. (2018). Application of ultrasound treatment for improving the physicochemical, functional and rheological

properties of myofibrillar proteins. *International journal of biological macromolecules, 111*, 139-147. doi: 10.1016/j.ijbiomac.2017.12.167

- Andreou, V., Dimopoulos, G., Alexandrakis, Z., Katsaros, G., Oikonomou, D., Toepfl, S., Heinz, V., Taoukis, P. (2017). Shelf-life evaluation of virgin olive oil extracted from olives subjected to nonthermal pretreatments for yield increase. *Innovative Food Science & Emerging Technologies, 40*, 52-57.
- Anjaneyulu, A., Sharma, N., & Kondaiah, N. (1990). Specific effect of phosphate on the functional properties and yield of buffalo meat patties. *Food chemistry, 36*(2), 149-154.
- AOAC 920.153 (1920). Association of Official Analytical Chemists (AOAC) official method 920.153. Ash of meat. Official methods of analysis of the AOAC international, Gaithersburg, Maryland: AOAC International.
- AOAC 991.43 (1994). Association of Official Analytical Chemists (AOAC) official method 991.43. Total, Soluble and Insoluble dietary fiber in foods. By Enzymatic-Gravimetric method, MES –TRIS buffer. Official methods of analysis of the AOAC international, Gaithersburg, Maryland: AOAC International.
- AOAC 2008.06 (2008). Association of Official Analytical Chemists (AOAC) official method 2008.06. Moisture and fat in meats. Microwave and nuclear magnetic renonance anlysis.Official methods of analysis of the AOAC international, Gaithersburg, Maryland: AOAC International.
- AOAC 925.10 (1925). Association of Official Analytical Chemists (AOAC) official method 925.10. Solids (total) and loss on drying (moisture) in flour. Air oven method. Official methods of analysis of the AOAC international, Gaithersburg, Maryland: AOAC International.
- AOAC 935.47 (1987). Association of Official Analytical Chemists (AOAC) official method 920.153. Salt (chlorine as sodium chloride) in meat. Volumetric method. Official methods of analysis of the AOAC international, Gaithersburg, Maryland: AOAC International.
- AOAC 936.07 (1936). Association of Official Analytical Chemists (AOAC) official method 936.07. Ash of flour. Magnesium acetate method. Official methods of analysis of the AOAC international, Gaithersburg, Maryland: AOAC International.
- AOAC 985.14 (1991). Association of Official Analytical Chemists (AOAC) official method 985.14. Moisture in Meat and Poultry Products – Rapid. By Microwave drying. Official methods of analysis of the AOAC international, Gaithersburg, Maryland: AOAC International.
- AOAC 985.15 (1991). Association of Official Analytical Chemists (AOAC) official method 985.15. Fat (Crude) in Meat and Poultry Products – Rapid. By Microwave drying. Official methods of analysis of the AOAC international, Gaithersburg, Maryland: AOAC International.
- AOAC 992.15 (1992). Association of Official Analytical Chemists (AOAC) official method 992.15. Crude protein in meat and meat products. Combustion method.

Official methods of analysis of the AOAC international, Gaithersburg, Maryland: AOAC International.

- Armitage, C. J., & Conner, M. (2001). Efficacy of the Theory of Planned Behaviour: A meta-analytic review. *British journal of social psychology, 40*(4), 471-499. doi: 10.1348/014466601164939
- Arzeni, C., Martínez, K., Zema, P., Arias, A., Pérez, O. E., & Pilosof, A. M. R. (2012). Comparative study of high intensity ultrasound effects on food proteins functionality. *Journal of Food Engineering, 108*(3), 463-472. doi: https://doi.org/10.1016/j.jfoodeng.2011.08.018
- Ashokkumar, M., Sunartio, D., Kentish, S., Mawson, R., Simons, L., Vilkhu, K., & Versteeg, C. (2008). Modification of food ingredients by ultrasound to improve functionality: A preliminary study on a model system. *Innovative food science & emerging technologies, 9*(2), 155-160. doi: 10.1016/j.ifset.2007.05.005
- Asif, M., Xuhui, W., Nasiri, A., & Ayyub, S. (2018). Determinant factors influencing organic food purchase intention and the moderating role of awareness: A comparative analysis. *Food quality and preference, 63*, 144-150. doi: 10.1016/j.foodqual.2017.08.006
- Asioli, D., Aschemann-Witzel, J., Caputo, V., Vecchio, R., Annunziata, A., Næs, T., & Varela, P. (2017). Making sense of the "clean label" trends: A review of consumer food choice behavior and discussion of industry implications. *Food Research International, 99*, 58-71. doi: https://doi.org/10.1016/j.foodres.2017.07.022
- Augusto, P. E. D., Ibarz, A., & Cristianini, M. (2013). Effect of high pressure homogenization (HPH) on the rheological properties of tomato juice: Viscoelastic properties and the Cox–Merz rule. *Journal of food engineering, 114*(1), 57-63. doi: 10.1016/j.jfoodeng.2012.07.025
- Authority, E. F. S. (2013). Assessment of one published review on health risks associated with phosphate additives in food. *EFSA Journal, 11*(11), 3444.
- Ayadi, M. A., Kechaou, A., Makni, I., & Attia, H. (2009). Influence of carrageenan addition on turkey meat sausages properties. *Journal of Food Engineering, 93*(3), 278-283. doi: https://doi.org/10.1016/j.jfoodeng.2009.01.033
- Aydar, A. Y. (2018). Utilization of response surface methodology in optimization of extraction of plant materials. Statistical approaches with emphasis on design of experiments applied to chemical processes, 157-169.
- Bajovic, B., Bolumar, T., & Heinz, V. (2012). Quality considerations with high pressure processing of fresh and value added meat products. *Meat Science, 92*(3), 280-289.
- Balasubramaniam, V. M., Barbosa-Cánovas, G. V., & Lelieveld, H. L. M. (2016). High-Pressure Processing Equipment for the Food Industry. In V. M. Balasubramaniam, G. V. Barbosa-Cánovas & H. L. M. Lelieveld (Eds.), *High*

Pressure Processing of Food: Principles, Technology and Applications (pp. 39-65). New York, NY: Springer New York.

- Balestra, F., & Petracci, M. (2019). Technofunctional ingredients for meat products: Current challenges *Sustainable meat production and processing* (pp. 45-68): Elsevier.
- Ballesteros, L. F., Teixeira, J. A., & Mussatto, S. I. (2014). Chemical, Functional, and Structural Properties of Spent Coffee Grounds and Coffee Silverskin. *Food and bioprocess technology, 7*(12), 3493-3503. doi: 10.1007/s11947-014-1349-z
- Banaś, A., Korus, A., & Korus, J. (2018). Texture, Color, and Sensory Features of Low-Sugar Gooseberry Jams Enriched with Plant Ingredients with Prohealth Properties. *Journal of food quality, 2018*, 1-12. doi: 10.1155/2018/1646894
- Bao, H. N. D., Ushio, H., & Ohshima, T. (2008). Antioxidative Activity and Antidiscoloration Efficacy of Ergothioneine in Mushroom (Flammulina velutipes) Extract Added to Beef and Fish Meats. *Journal of agricultural and food chemistry, 56*(21), 10032-10040. doi: 10.1021/jf8017063
- Baptista, I., Rocha, S. M., Cunha, A., Saraiva, J. A., & Almeida, A. (2016). Inactivation of Staphylococcus aureus by high pressure processing: An overview. *Innovative Food Science & Emerging Technologies, 36*, 128-149.
- Barbut, S., Wood, J., & Marangoni, A. (2016). Quality effects of using organogels in breakfast sausage. *Meat Science, 122*, 84-89. doi: 10.1016/j.meatsci.2016.07.022
- Basha, M. B., & Lal, D. (2019). Indian consumers' attitudes towards purchasing organically produced foods: An empirical study. *Journal of cleaner production, 215*, 99-111. doi: 10.1016/j.jclepro.2018.12.098
- Baublits, R., Pohlman, F., Brown Jr, A., & Johnson, Z. (2005). Effects of enhancement with varying phosphate types and concentrations, at two different pump rates on beef biceps femoris instrumental color characteristics. *Meat Science, 71*(2), 264-276.
- Baublits, R., Pohlman, F., Brown Jr, A., & Johnson, Z. (2006). Effects of enhancement with differing phosphate types, concentrations, and pump rates, without sodium chloride, on beef biceps femoris instrumental color characteristics. *Meat Science, 72*(3), 503-512.
- Baugreet, S., Kerry, J. P., Allen, P., & Hamill, R. M. (2017). Optimisation of protein-fortified beef patties targeted to the needs of older adults: a mixture design approach. *Meat Science, 134*, 111-118. doi: 10.1016/j.meatsci.2017.07.023
- Bearth, A., Cousin, M.-E., & Siegrist, M. (2014). The consumer's perception of artificial food additives: Influences on acceptance, risk and benefit perceptions. *Food quality and preference, 38*, 14-23. doi: 10.1016/j.foodqual.2014.05.008

- Bedale, W., Sindelar, J. J., & Milkowski, A. L. (2016). Dietary nitrate and nitrite: Benefits, risks, and evolving perceptions. *Meat Science, 120*, 85-92. doi: 10.1016/j.meatsci.2016.03.009
- Belmiro, R. H., Oliveira, L. d. C., Tribst, A. A. L., & Cristianini, M. (2022). Techno-functional properties of coffee by-products are modified by dynamic high pressure: A case study of clean label ingredient in cookies. *Food science & technology, 154*, 112601. doi: 10.1016/j.lwt.2021.112601
- Beltran, E., Pla, R., Yuste, J., & Mor-Mur, M. (2003). Lipid oxidation of pressurized and cooked chicken: role of sodium chloride and mechanical processing on TBARS and hexanal values. *Meat Science, 64*(1), 19-25. doi: 10.1016/S0309-1740(02)00132-8
- Bengtsson, H., & Tornberg, E. V. A. (2011). Physicochemical characterization of fruit and vegetable fiber suspensions. I: effect of homogenization. *Journal of texture studies, 42*(4), 268-280. doi: 10.1111/j.1745-4603.2010.00275.x
- Bertolino, M., Barbosa-Pereira, L., Ghirardello, D., Botta, C., Rolle, L., Guglielmetti, A., Borotto Dalla Vechhia, S., & Zeppa, G. (2019). Coffee silverskin as nutraceutical ingredient in yogurt: its effect on functional properties and its bioaccessibility. *Journal of the science of food and agriculture, 99*(9), 4267-4275. doi: 10.1002/jsfa.9659
- Bertram, H. C., Aaslyng, M. D., & Andersen, H. J. (2005). Elucidation of the relationship between cooking temperature, water distribution and sensory attributes of pork – a combined NMR and sensory study. *Meat Science, 70*(1), 75-81. doi: 10.1016/j.meatsci.2004.12.002
- Bertram, H. C., Andersen, H. J., & Karlsson, A. H. (2001). Comparative study of low-field NMR relaxation measurements and two traditional methods in the determination of water holding capacity of pork. *Meat Science, 57*(2), 125-132.
- Bertram, H. C., Meyer, R. L., Wu, Z., Zhou, X., & Andersen, H. J. (2008). Water distribution and microstructure in enhanced pork. *Journal of Agricultural and Food Chemistry, 56*(16), 7201-7207.
- Bertram, H. C., Purslow, P. P., & Andersen, H. J. (2002). Relationship between Meat Structure, Water Mobility, and Distribution: A Low-Field Nuclear Magnetic Resonance Study. *Journal of agricultural and food chemistry, 50*(4), 824-829. doi: 10.1021/jf010738f
- Bertram, H. C., Schäfer, A., Rosenvold, K., & Andersen, H. J. (2004). Physical changes of significance for early post mortem water distribution in porcine M. longissimus. *Meat Science, 66*(4), 915-924. doi: 10.1016/S0309-1740(03)00188-8
- Borrelli, R. C., Esposito, F., Napolitano, A., Ritieni, A., & Fogliano, V. (2004). Characterization of a New Potential Functional Ingredient: Coffee Silverskin. *Journal of agricultural and food chemistry, 52*(5), 1338-1343. doi: 10.1021/jf034974x

- Botsoglou, N. A., Fletouris, D. J., Papageorgiou, G. E., Vassilopoulos, V. N., Mantis, A. J., & Trakatellis, A. G. (1994). Rapid, sensitive, and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food, and feedstuff samples. *Journal of Agricultural and Food Chemistry, 42*(9), 1931-1937.
- Bourne, M. C. (1978). Texture profile analysis. *Food Technology, 32*, 62-66.
- Boyce, C., & Neale, P. (2006). Conducting in-depth interviews: A guide for designing and conducting in-depth interviews for evaluation input.
- Byrne, F., Gillman, B., E, O. R., Smith, O., Cronin, I., & Eustace, J. (2021). European food safety guidelines on dietary phosphate additives: implications for people with chronic kidney disease. Ir J Med Sci, 190(4), 1655-1657. doi: 10.1007/s11845-020-02497-9
- Byrne, F. N., Gillman, B., Kiely, M., Bowles, M., Connolly, P., Earlie, J., Murphy, J., Rennick, T., O'Reilly, E., Shiely, F., Kearney, P., & Eustace, J. (2021). Revising Dietary Phosphorus Advice in Chronic Kidney Disease G3-5D. Journal of Renal Nutrition, 31(2), 132-143. doi: https://doi.org/10.1053/j.jrn.2020.04.003
- Calvete-Torre, I., Muñoz-Almagro, N., Pacheco, M. T., Antón, M. J., Dapena, E., Ruiz, L., Margolles, A., Villamiel, M., Moreno, F. J. (2021). Apple pomaces derived from mono-varietal Asturian ciders production are potential source of pectins with appealing functional properties. *Carbohydrate polymers, 264*, 117980-117980. doi: 10.1016/j.carbpol.2021.117980
- Calvo, M. S., & Uribarri, J. (2013). Public health impact of dietary phosphorus excess on bone and cardiovascular health in the general population. *Am J Clin Nutr, 98*(1), 6-15. doi: 10.3945/ajcn.112.053934
- Câmara, A. K. F. I., Okuro, P. K., Santos, M., Paglarini, C. d. S., da Cunha, R. L., Ruiz-Capillas, C., Herrero, A. M., & Pollonio, M. A. R. (2020). Understanding the role of chia (Salvia Hispanica L.) mucilage on olive oil-based emulsion gels as a new fat substitute in emulsified meat products. *European food research & technology, 246*(5), 909-922. doi: 10.1007/s00217-020-03457-4
- Câmara, A. K. F. I., Vidal, V. A. S., Santos, M., Bernardinelli, O. D., Sabadini, E., & Pollonio, M. A. R. (2020). Reducing phosphate in emulsified meat products by adding chia (Salvia hispanica L.) mucilage in powder or gel format: A clean label technological strategy. *Meat Science, 163*, 108085-108085. doi: 10.1016/j.meatsci.2020.108085
- Campo, M. M., Nute, G. R., Hughes, S. I., Enser, M., Wood, J. D., & Richardson, R. I. (2006). Flavour perception of oxidation in beef. *Meat Science, 72*(2), 303-311. doi: 10.1016/j.meatsci.2005.07.015
- Carfora, V., Caso, D., & Conner, M. (2017). Correlational study and randomised controlled trial for understanding and changing red meat consumption: The role of eating identities. *Social science & medicine (1982), 175*, 244-252. doi: 10.1016/j.socscimed.2017.01.005

- Carfora, V., Cavallo, C., Caso, D., Del Giudice, T., De Devitiis, B., Viscecchia, R., Nardone, G., & Cicia, G. (2019). Explaining consumer purchase behavior for organic milk: Including trust and green self-identity within the theory of planned behavior. *Food quality and preference, 76*, 1-9. doi: 10.1016/j.foodqual.2019.03.006
- Carocho, M., Morales, P., & Ferreira, I. C. F. R. (2015). Natural food additives: Quo vadis? *Trends in Food Science & Technology, 45*(2), 284-295. doi: https://doi.org/10.1016/j.tifs.2015.06.007
- Carvalho, L. T., Pires, M. A., Baldin, J. C., Munekata, P. E. S., de Carvalho, F. A. L., Rodrigues, I., Polizer, Y. J., de Mello, J. L. M., Lapa-Guimarães, J., & Trindade, M. A. (2019). Partial replacement of meat and fat with hydrated wheat fiber in beef burgers decreases caloric value without reducing the feeling of satiety after consumption. *Meat Science, 147*, 53-59. doi: 10.1016/j.meatsci.2018.08.010
- Casco, G., Veluz, G., & Alvarado, C. (2013). SavorPhos as an all-natural phosphate replacer in water-and oil-based marinades for rotisserie birds and boneless-skinless breast. *Poultry Science, 92*(12), 3236-3243.
- Cava, R., Higuero, N., & Ladero, L. (2021). High-pressure processing and storage temperature on Listeria monocytogenes, microbial counts and oxidative changes of two traditional dry-cured meat products. *Meat Science, 171*, 108273-108273. doi: 10.1016/j.meatsci.2020.108273
- Chandrapala, J., Martin, G. J. O., Zisu, B., Kentish, S. E., & Ashokkumar, M. (2012). The effect of ultrasound on casein micelle integrity. *Journal of dairy science, 95*(12), 6882-6890. doi: 10.3168/jds.2012-5318
- Chang, H.-J., Wang, Q., Tang, C.-H., & Zhou, G.-H. (2015). Effects of Ultrasound Treatment on Connective Tissue Collagen and Meat Quality of Beef Semitendinosus Muscle. *Journal of food quality, 38*(4), 256-267. doi: 10.1111/jfq.12141
- Chen, B., Cai, Y., Liu, T., Huang, L., Deng, X., Zhao, Q., & Zhao, M. (2019). Improvements in physicochemical and emulsifying properties of insoluble soybean fiber by physical-chemical treatments. *Food hydrocolloids, 93*, 167-175. doi: 10.1016/j.foodhyd.2019.01.058
- Chen, M.-F. (2017). Modeling an extended theory of planned behavior model to predict intention to take precautions to avoid consuming food with additives. *Food quality and preference, 58*, 24-33. doi: 10.1016/j.foodqual.2017.01.002
- Chen, M.-F., & Tung, P.-J. (2014). Developing an extended Theory of Planned Behavior model to predict consumers' intention to visit green hotels. *International journal of hospitality management, 36*, 221-230. doi: 10.1016/j.ijhm.2013.09.006
- Chen, X., Tume, R. K., Xiong, Y., Xu, X., Zhou, G., Chen, C., & Nishiumi, T. (2018). Structural modification of myofibrillar proteins by high-pressure processing for

functionally improved, value-added, and healthy muscle gelled foods. *Critical Reviews in Food Science and Nutrition, 58*(17), 2981-3003.

- Choe, J., Lee, J., Jo, K., Jo, C., Song, M., & Jung, S. (2018). Application of winter mushroom powder as an alternative to phosphates in emulsion-type sausages. *Meat Science, 143*, 114-118. doi: 10.1016/j.meatsci.2018.04.038
- Choi, Y.-S., Choi, J.-H., Han, D.-J., Kim, H.-Y., Kim, H.-W., Lee, M.-A., Chung, H-J., & Kim, C.-J. (2012). Effects of Laminaria japonica on the physico-chemical and sensory characteristics of reduced-fat pork patties. *Meat Science, 91*(1), 1-7. doi: 10.1016/j.meatsci.2011.11.011
- Choi, Y.-S., Kim, Y.-B., Hwang, K.-E., Song, D.-H., Ham, Y.-K., Kim, H.-W., Sung, J-M., & Kim, C.-J. (2016). Effect of apple pomace fiber and pork fat levels on quality characteristics of uncured, reduced-fat chicken sausages. *Poultry Science, 95*(6), 1465-1471. doi: 10.3382/ps/pew096
- Choi, Y.-S., Park, K.-S., Kim, H.-W., Hwang, K.-E., Song, D.-H., Choi, M.-S., Lee, S-Y., Paik, H-D., & Kim, C.-J. (2013). Quality characteristics of reduced-fat frankfurters with pork fat replaced by sunflower seed oils and dietary fiber extracted from makgeolli lees. *Meat Science, 93*(3), 652-658. doi: 10.1016/j.meatsci.2012.11.025
- Cichoski, A. J., Silva, M. S., Leães, Y. S. V., Brasil, C. C. B., de Menezes, C. R., Barin, J. S., Wagner, R., & Campagnol, P. C. B. (2019). Ultrasound: A promising technology to improve the technological quality of meat emulsions. *Meat Science, 148*, 150-155. doi: 10.1016/j.meatsci.2018.10.009
- Codex Standard 192. (1995). In *General standard for food additives*: Food and Agricultural Organisation of the United Nations.
- Conroy, P. M., O'Sullivan, M. G., Hamill, R. M., & Kerry, J. P. (2018). Impact on the physical and sensory properties of salt-and fat-reduced traditional Irish breakfast sausages on various age cohorts acceptance. *Meat Science, 143*, 190-198. doi: 10.1016/j.meatsci.2018.04.037
- Cornélio, M. E., Gallani, M. C. B. J., Godin, G., Rodrigues, R. C. M., Nadruz, W., & Mendez, R. D. R. (2012). Behavioural determinants of salt consumption among hypertensive individuals: Behavioural determinants of salt consumption. *Journal of human nutrition and dietetics, 25*(4), 334-344. doi: 10.1111/j.1365-277X.2012.01238.x
- Cox, S., & Abu-Ghannam, N. (2013). Enhancement of the phytochemical and fibre content of beef patties with Himanthalia elongata seaweed. *International journal of food science & technology, 48*(11), 2239-2249. doi: 10.1111/ijfs.12210
- Crehan, C. M., Troy, D. J., & Buckley, D. J. (2000). Effects of salt level and high hydrostatic pressure processing on frankfurters formulated with 1.5 and 2.5% salt. *Meat Science, 55*(1), 123-130. doi: 10.1016/S0309-1740(99)00134-5
- Cronbach, L. J. (1951). Coefficient alpha and the internal structure of tests. *psychometrika, 16*(3), 297-334.

- Cruz-Romero, M. C., O'Flynn, C. C., Troy, D., Mullen, A. M., & Kerry, J. P. (2022). The Use of Potassium Chloride and Tapioca Starch to Enhance the Flavour and Texture of Phosphate- and Sodium-Reduced Low Fat Breakfast Sausages Manufactured Using High Pressure-Treated Meat. Foods, 11(1), 17. https://www.mdpi.com/2304-8158/11/1/17
- Das, A. K., Nanda, P. K., Madane, P., Biswas, S., Das, A., Zhang, W., & Lorenzo, J. M. (2020). A comprehensive review on antioxidant dietary fibre enriched meat-based functional foods. *Trends in food science & technology, 99*, 323-336. doi: 10.1016/j.tifs.2020.03.010
- Delgado-Pando, G., Ekonomou, S. I., Stratakos, A. C., & Pintado, T. (2021). Clean Label Alternatives in Meat Products. *Foods, 10*(7), 1615. doi: 10.3390/foods10071615
- DeVries, J., Camire, M., Cho, S., Craig, S., Gordon, D., Jones, J., Li, B., Lineback, D., Prosky, L., & Tungland, B. (2001). The definition of dietary fiber. *Cereal Foods World, 46*(3), 112-129.
- Ding, L., Liu, M., Yang, Y., & Ma, W. (2022). Understanding Chinese consumers' purchase intention towards traceable seafood using an extended Theory of Planned Behavior model. *Marine policy, 137*. doi: 10.1016/j.marpol.2022.104973
- Domingo, J. L., & Nadal, M. (2017). Carcinogenicity of consumption of red meat and processed meat: A review of scientific news since the IARC decision. *Food and chemical toxicology, 105*, 256-261. doi: 10.1016/j.fct.2017.04.028
- Dorce, L. C., da Silva, M. C., Mauad, J. R. C., de Faria Domingues, C. H., & Borges, J. A. R. (2021). Extending the theory of planned behavior to understand consumer purchase behavior for organic vegetables in Brazil: The role of perceived health benefits, perceived sustainability benefits and perceived price. *Food quality and preference, 91*, 104191. doi: 10.1016/j.foodqual.2021.104191
- Dykes, G. A., Coorey, R., Ravensdale, J. T., & Sarjit, A. (2019). Phosphates. In L. Melton, F. Shahidi, & P. Varelis (Eds.), *Encyclopedia of food chemistry* (pp. 218-224). Oxford: Academic Press.
- El Zeny, T., Essa, R. Y., Bisar, B. A., & Metwalli, S. (2019). Effect of using chicory roots powder as a fat replacer on beef burger quality. *Slovenian Veterinary Research, 56*(Suppl. 22), 509-514.
- Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C., & Attia, H. (2011). Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. *Food chemistry, 124*(2), 411-421. doi: 10.1016/j.foodchem.2010.06.077
- Elliott, M. A., Armitage, C. J., & Baughan, C. J. (2007). Using the theory of planned behaviour to predict observed driving behaviour. *British journal of social psychology, 46*(1), 69-90. doi: 10.1348/014466605X90801
- Erdogdu, S. B., Erdogdu, F., & Ekiz, H. I. (2007). Influence of sodium tripolyphosphate (stp) treatment and cooking time on cook losses and textural

properties of red meats. *30*(6), 685-700. Retrieved from https://go.exlibris.link/5pF3cTSK doi:10.1111/j.1745-4530.2007.00139.x

- Fan, X., Chang, H., Lin, Y., Zhao, X., Zhang, A., Li, S., Feng, Z., & Chen, X. (2020). Effects of ultrasound-assisted enzyme hydrolysis on the microstructure and physicochemical properties of okara fibers. *Ultrasonics sonochemistry, 69*, 105247-105247. doi: 10.1016/j.ultsonch.2020.105247

- Fang, Z., Lin, P., Ha, M., & Warner, R. D. (2019). Effects of incorporation of sugarcane fibre on the physicochemical and sensory properties of chicken sausage. *International journal of food science & technology, 54*(4), 1036-1044. doi: 10.1111/ijfs.13894

- Feiner, G. (2006). *Meat products handbook: Practical science and technology*: Elsevier.

- Fellendorf, S., Kerry, J. P., & O'Sullivan, M. G. (2018). Consumer attitudes on salt and fat reduced foods in the Republic of Ireland. doi: 10.4236/fns.2018.97066

- Fernández-López, J., Sayas-Barberá, E., Pérez-Alvarez, J., & Aranda-Catalá, V. (2004). Effect of sodium chloride, sodium tripolyphosphate and pH on color properties of pork meat. *Color Research & Application: Endorsed by Inter-Society Color Council, The Colour Group (Great Britain), Canadian Society for Color, Color Science Association of Japan, Dutch Society for the Study of Color, The Swedish Colour Centre Foundation, Colour Society of Australia, Centre Français de la Couleur, 29*(1), 67-74.

- Figuerola, F., Hurtado, M. a. L., Estévez, A. M. a., Chiffelle, I., & Asenjo, F. (2005). Fibre concentrates from apple pomace and citrus peel as potential fibre sources for food enrichment. *Food chemistry, 91*(3), 395-401. doi: 10.1016/j.foodchem.2004.04.036

- Fishbein, M., & Ajzen, I. (1975). *Belief, attitude, intention, and behavior: an introduction to theory and research*. Reading, Mass: Addison-Wesley Pub. Co.

- Francis, J., Eccles, M. P., Johnston, M., Walker, A., Grimshaw, J. M., Foy, R., Kaner, E. F. S., Smith, L., & Bonetti, D. (2004). Constructing questionnaires based on the theory of planned behaviour: A manual for health services researchers.

- Fuentes, V., Ventanas, J., Morcuende, D., Estévez, M., & Ventanas, S. (2010). Lipid and protein oxidation and sensory properties of vacuum-packaged dry-cured ham subjected to high hydrostatic pressure. *Meat Science, 85*(3), 506-514. doi: 10.1016/j.meatsci.2010.02.024

- Gabiatti, C., Neves, I. C. O., Lim, L.-T., Bohrer, B. M., Rodrigues, R. C., & Prentice, C. (2020). Characterization of dietary fiber from residual cellulose sausage casings using a combination of enzymatic treatment and high-speed homogenization. *Food hydrocolloids, 100*, 105398. doi: 10.1016/j.foodhyd.2019.105398

- García-Pérez, J. V., Cárcel, J. A., Benedito, J., & Mulet, A. (2007). Power Ultrasound Mass Transfer Enhancement in Food Drying. *Food and bioproducts processing, 85*(3), 247-254. doi: 10.1205/fbp07010

- Garcia-Serna, E., Martinez-Saez, N., Mesias, M., Morales, F. J., & Castillo, M. D. d. (2014). Use of Coffee Silverskin and Stevia to Improve the Formulation of Biscuits. *Polish journal of food and nutrition sciences, 64*(4), 243-251. doi: 10.2478/pjfns-2013-0024

- Garcia-Vaquero, M., Lopez-Alonso, M., & Hayes, M. (2017). Assessment of the functional properties of protein extracted from the brown seaweed Himanthalia elongata (Linnaeus) S. F. Gray. *Food research international, 99*(Pt 3), 971-978. doi: 10.1016/j.foodres.2016.06.023

- Gemechu, F. G. (2020). Embracing nutritional qualities, biological activities and technological properties of coffee byproducts in functional food formulation. *Trends in food science & technology, 104*, 235-261. doi: 10.1016/j.tifs.2020.08.005

- Genccelep, H., Saricaoglu, F. T., Anil, M., Agar, B., & Turhan, S. (2015). The effect of starch modification and concentration on steady-state and dynamic rheology of meat emulsions. *Food Hydrocolloids, 48*, 135-148.

- Glorieux, S., Goemaere, O., Steen, L., & Fraeye, I. (2017). Phosphate reduction in emulsified meat products: Impact of phosphate type and dosage on quality characteristics. *Food Technology and Biotechnology, 55*(3), 390.

- Grønhøj, A., Bech-Larsen, T., Chan, K., & Tsang, L. (2012). Using theory of planned behavior to predict healthy eating among Danish adolescents. *Health education (Bradford, West Yorkshire, England), 113*(1), 4-17. doi: 10.1108/09654281311293600

- Grossi, A., Olsen, K., Bolumar, T., Rinnan, Å., Øgendal, L. H., & Orlien, V. (2016). The effect of high pressure on the functional properties of pork myofibrillar proteins. *Food chemistry, 196*, 1005-1015. doi: 10.1016/j.foodchem.2015.10.062

- Grossi, A., Søltoft-Jensen, J., Knudsen, J. C., Christensen, M., & Orlien, V. (2011). Synergistic cooperation of high pressure and carrot dietary fibre on texture and colour of pork sausages. *Meat Science, 89*(2), 195-201. doi: 10.1016/j.meatsci.2011.04.017

- Grossi, A., Søltoft-Jensen, J., Knudsen, J. C., Christensen, M., & Orlien, V. (2012). Reduction of salt in pork sausages by the addition of carrot fibre or potato starch and high pressure treatment. *Meat Science, 92*(4), 481-489. doi: 10.1016/j.meatsci.2012.05.015

- Guàrdia, M. D., Guerrero, L., Gelabert, J., Gou, P., & Arnau, J. (2006). Consumer attitude towards sodium reduction in meat products and acceptability of fermented sausages with reduced sodium content. *Meat Science, 73*(3), 484-490. doi: 10.1016/j.meatsci.2006.01.009

- Guglielmetti, A., Fernandez-Gomez, B., Zeppa, G., & Del Castillo, M. D. (2019). Nutritional Quality, Potential Health Promoting Properties and Sensory Perception of an Improved Gluten-Free Bread Formulation Containing Inulin, Rice Protein and Bioactive Compounds Extracted from Coffee Byproducts. *Polish*

journal of food and nutrition sciences, 69(2), 157-166. doi: 10.31883/pjfns-2019-0012

- Gyawali, R., & Ibrahim, S. A. (2016). Effects of hydrocolloids and processing conditions on acid whey production with reference to Greek yogurt. *Trends in food science & technology, 56*, 61-76. doi: 10.1016/j.tifs.2016.07.013
- Han, M., & Bertram, H. C. (2017). Designing healthier comminuted meat products: Effect of dietary fibers on water distribution and texture of a fat-reduced meat model system. *Meat Science, 133*, 159-165. doi: 10.1016/j.meatsci.2017.07.001
- Han, M., Wang, P., Xu, X., & Zhou, G. (2014). Low-field NMR study of heat-induced gelation of pork myofibrillar proteins and its relationship with microstructural characteristics. *Food research international, 62*, 1175-1182. doi: 10.1016/j.foodres.2014.05.062
- Handal, M. N., Rohde, J. F., Jacobsen, R., & Heitmann, B, L. (2021). Processed meat consumption and the Risk of cance: A critical evaluation of the constraints of current evidence from epidemiological studies. *Nutrients, 13*(10), 3601. doi:10.3390/nu13103601
- Hansen, J., Sparleanu, C., Liang, Y., Büchi, J., Bansal, S., Caro, M. Á., & Staedtler, F. (2021). Exploring cultural concepts of meat and future predictions on the timeline of cultured meat. *Future Foods, 4*, 100041.
- Hathwar, S. C., Rai, A. K., Modi, V. K., & Narayan, B. (2011). Characteristics and consumer acceptance of healthier meat and meat product formulations—a review. *Journal of food science and technology, 49*(6), 653-664. doi: 10.1007/s13197-011-0476-z
- Heinz, V. (2014). Development of shock wave technology for packed meat. Shockmeat EU 7th Framework Programme. Grant Agreement number: 287034.
- Henchion, M., & Zimmermann, J. (2021). Animal food products: policy, market and social issues and their influence on demand and supply of meat. *Proceedings of the Nutrition Society, 80*(2), 252-263. doi: 10.1017/S0029665120007971
- Henchion, M., Moloney, A. P., Hyland, J., Zimmermann, J., & McCarthy, S. (2021). Review: Trends for meat, milk and egg consumption for the next decades and the role played by livestock systems in the global production of proteins. *Animal (Cambridge, England), 15*, 100287-100287. doi: 10.1016/j.animal.2021.100287
- Henning, S. S. C., Tshalibe, P., & Hoffman, L. C. (2016). Physico-chemical properties of reduced-fat beef species sausage with pork back fat replaced by pineapple dietary fibres and water. *Food science & technology, 74*, 92-98. doi: 10.1016/j.lwt.2016.07.007
- Hernández-Hernández, H. M., Moreno-Vilet, L., & Villanueva-Rodríguez, S. J. (2019). Current status of emerging food processing technologies in Latin America: Novel non-thermal processing. *Innovative food science & emerging technologies, 58*, 102233. doi: 10.1016/j.ifset.2019.102233

- Hruska, K. A., Mathew, S., Lund, R., Qiu, P., & Pratt, R. (2008). Hyperphosphatemia of chronic kidney disease. *Kidney international, 74*(2), 148-157. doi: 10.1038/ki.2008.130
- Hu, H., Wu, J., Li-Chan, E. C. Y., Zhu, L., Zhang, F., Xu, X., Fan, G., Wang, L., Huang, X., & Pan, S. (2013). Effects of ultrasound on structural and physical properties of soy protein isolate (SPI) dispersions. *Food hydrocolloids, 30*(2), 647-655. doi: 10.1016/j.foodhyd.2012.08.001
- Huang, H.-W., Wu, S.-J., Lu, J.-K., Shyu, Y.-T., & Wang, C.-Y. (2017). Current status and future trends of high-pressure processing in food industry. *Food Control, 72*, 1-8. doi: https://doi.org/10.1016/j.foodcont.2016.07.019
- Hughes, E., Mullen, A. M., & Troy, D. J. (1998). Effects of fat level, tapioca starch and whey protein on frankfurters formulated with 5% and 12% fat. *Meat Science, 48*(1), 169-180. doi: 10.1016/S0309-1740(97)00087-9
- Hussain, S., Sharma, M., & Bhat, R. (2021). Valorisation of sea buckthorn pomace by optimization of ultrasonic-assisted extraction of soluble dietary fibre using response surface methodology. *Foods, 10*(6), 1330.
- Hygreeva, D., & Pandey, M. C. (2016). Novel approaches in improving the quality and safety aspects of processed meat products through high pressure processing technology - A review. *Trends in food science & technology, 54*, 175-185. doi: 10.1016/j.tifs.2016.06.002
- Ikeuchi, Y., Tanji, H., Kim, K., & Suzuki, A. (1992). Dynamic rheological measurements on heat-induced pressurized actomyosin gels. *Journal of Agricultural and Food Chemistry, 40*(10), 1751-1755.
- Illippangama, A. U., Jayasena, D. D., Jo, C., & Mudannayake, D. C. (2022). Inulin as a functional ingredient and their applications in meat products. *Carbohydrate polymers, 275*, 118706-118706. doi: 10.1016/j.carbpol.2021.118706
- Ingredion (2014). The Clean label guide in Europe. Retrieved from http://www.alimentatec.com/wp-content/uploads/2014/10/The-Clean-Label-Guide-To-Europe.pdf
- Inguglia, E. S., Zhang, Z., Burgess, C., Kerry, J. P., & Tiwari, B. K. (2018). Influence of extrinsic operational parameters on salt diffusion during ultrasound assisted meat curing. *Ultrasonics, 83*, 164-170. doi: 10.1016/j.ultras.2017.03.017
- Inguglia, E. S., Zhang, Z., Tiwari, B. K., Kerry, J. P., & Burgess, C. M. (2017). Salt reduction strategies in processed meat products – A review. *Trends in food science & technology, 59*, 70-78. doi: 10.1016/j.tifs.2016.10.016
- Iriondo-DeHond, A., Aparicio García, N., Fernandez-Gomez, B., Guisantes-Batan, E., Velázquez Escobar, F., Blanch, G. P., San Andres, M. I., Sanchez-Fortun, S., & del Castillo, M. D. (2019). Validation of coffee by-products as novel food ingredients. *Innovative food science & emerging technologies, 51*, 194-204. doi: 10.1016/j.ifset.2018.06.010

- Irish Universities Nutrition Alliance (IUNA) (2011). National adult nutrition survey (NANS) summary report, p. 37.
- Jamalabadi, M., Saremnezhad, S., Bahrami, A., & Jafari, S. M. (2019). The influence of bath and probe sonication on the physicochemical and microstructural properties of wheat starch. *Food science & nutrition, 7*(7), 2427-2435. doi: 10.1002/fsn3.1111
- Jiang, J., & Xiong, Y. L. (2015). Role of interfacial protein membrane in oxidative stability of vegetable oil substitution emulsions applicable to nutritionally modified sausage. *Meat Science, 109*, 56-65. doi: 10.1016/j.meatsci.2015.05.011
- Jung, S., Nam, K. C., Ahn, D. U., Kim, H. J., & Jo, C. (2013). Effect of phosvitin on lipid and protein oxidation in ground beef treated with high hydrostatic pressure. *Meat Science, 95*(1), 8-13. doi: 10.1016/j.meatsci.2013.04.005
- Kalantar-Zadeh, K., Gutekunst, L., Mehrotra, R., Kovesdy, C. P., Bross, R., Shinaberger, C. S., Noori, N., Hirschberg, R., Benner, D., Nissenson, A. R., & Kopple, J. D. (2010). Understanding sources of dietary phosphorus in the treatment of patients with chronic kidney disease. *Clin J Am Soc Nephrol, 5*(3), 519-530. doi: 10.2215/cjn.06080809
- Kang, D., Jiang, Y., Xing, L., Zhou, G., & Zhang, W. (2017). Inactivation of Escherichia coli O157:H7 and Bacillus cereus by power ultrasound during the curing processing in brining liquid and beef. *Food research international, 102*, 717-727. doi: 10.1016/j.foodres.2017.09.062
- Kang, D.-c., Gao, X.-q., Ge, Q.-f., Zhou, G.-h., & Zhang, W.-g. (2017). Effects of ultrasound on the beef structure and water distribution during curing through protein degradation and modification. *Ultrasonics sonochemistry, 38*, 317-325. doi: 10.1016/j.ultsonch.2017.03.026
- Keenan, D. F., Resconi, V. C., Kerry, J. P., & Hamill, R. M. (2014). Modelling the influence of inulin as a fat substitute in comminuted meat products on their physico-chemical characteristics and eating quality using a mixture design approach. *Meat Science, 96*(3), 1384-1394. doi: 10.1016/j.meatsci.2013.11.025
- Khouryieh, H. A. (2021). Novel and emerging technologies used by the U.S. food processing industry. *Innovative food science & emerging technologies, 67*, 102559. doi: 10.1016/j.ifset.2020.102559
- Kim, H.-W., Miller, D. K., Lee, Y. J., & Kim, Y. H. B. (2016). Effects of soy hull pectin and insoluble fiber on physicochemical and oxidative characteristics of fresh and frozen/thawed beef patties. *Meat Science, 117*, 63-67. doi: 10.1016/j.meatsci.2016.02.035
- Kiran, M., Nagabhushan, C., Prabhu, N. K., Paramesha, S. C., Rajshekar, T., Praveen, M. P., Punitkumar, C., Puneetha, S. C., Kumar, & R., Rahul, Y. (2018). Consumption pattern, consumer attitude and consumer perception on meat quality and safety in Southern India. *International food research journal, 25*(3), 1026-1030.

- Knight, P., & Parsons, N. (1988). Action of NaCl and polyphosphates in meat processing: Responses of myofibrils to concentrated salt solutions. *Meat Science, 24*(4), 275-300.
- Köhn, C. R., Almeida, J. C., Schmidt, M. M., Vidal, A. R., Kempka, A. P., Demiate, I. M., Rosa, C. S., Kubota, E. H., Mello, R. O., & Prestes, R. C. (2016). Evaluation of water absorption capacity of ingredients and additives used in the meat industry submitted to different saline concentrations and ultrasound. *International food research journal, 23*(2), 653.
- Koyratty, B. N. S., Aumjaud, B., & Amnee Neeliah, S. (2014). Food additive control: a survey among selected consumers and manufacturers. *British food journal (1966), 116*(2), 353-372. doi: 10.1108/BFJ-05-2012-0125
- Kurt, A., & Gençcelep, H. (2018). Enrichment of meat emulsion with mushroom (Agaricus bisporus) powder: Impact on rheological and structural characteristics. *Journal of food engineering, 237*, 128-136. doi: 10.1016/j.jfoodeng.2018.05.028
- Lampila, L. E. (2013). Applications and functions of food-grade phosphates. *Annals of the New York Academy of Sciences, 1301*(1), 37-44. doi: 10.1111/nyas.12230
- Lampila, L. E., & Godber, J. P. (2002). Food Phospates. In P. M. D. A. Larry Branen, Seppo Salminen, John H. Thorngate III (Ed.), *Food Additives* (second ed., pp. 809-898). New York: Marcel Dekker Inc.
- Lampila, L. E., & McMillin, K. W. (2017). Phosphorus additives in food processing *Clinical aspects of natural and added phosphorus in foods* (pp. 99-110): Springer.
- Lamsal, B. P., & Faubion, J. M. (2009). The Beneficial Use of Cereal and Cereal Components in Probiotic Foods. *Food reviews international, 25*(2), 103-114. doi: 10.1080/87559120802682573
- LeMaster, M. N., Chauhan, S. S., Wick, M. P., Clark, D. L., & England, E. M. (2019). Potassium carbonate improves fresh pork quality characteristics. *Meat Science, 156*, 222-230. doi: 10.1016/j.meatsci.2019.05.019
- León, J. B., Sullivan, C. M., & Sehgal, A. R. (2013). The prevalence of phosphorus-containing food additives in top-selling foods in grocery stores. *Journal of renal nutrition : the official journal of the Council on Renal Nutrition of the National Kidney Foundation, 23*(4), 265-270.e262. doi: 10.1053/j.jrn.2012.12.003
- Li, C., Liu, D., Zhou, G., Xu, X., Qi, J., Shi, P., & Xia, T. (2012). Meat quality and cooking attributes of thawed pork with different low field NMR T21. *Meat Science, 92*(2), 79-83. doi: 10.1016/j.meatsci.2011.11.015
- Lianji, M., & Chen, N. (1989). *Research in improving the water holding capacity (WHC) of meat in sausage products.* Paper presented at the ICoMST.
- Lim, H.-R., & An, S. (2021). Intention to purchase wellbeing food among Korean consumers: An application of the Theory of Planned Behavior. *Food quality and preference, 88*, 104101-104101. doi: 10.1016/j.foodqual.2020.104101

- Liu, Y., Yi, S., Ye, T., Leng, Y., Alomgir Hossen, M., Sameen, D. E., Dai, J., Li, S., & Qin, W. (2021). Effects of ultrasonic treatment and homogenization on physicochemical properties of okara dietary fibers for 3D printing cookies. *Ultrasonics sonochemistry, 77*, 105693-105693. doi: 10.1016/j.ultsonch.2021.105693

- Llauger, M., Claret, A., Bou, R., López-Mas, L., & Guerrero, L. (2021). Consumer Attitudes toward Consumption of Meat Products Containing Offal and Offal Extracts. *Foods, 10*(7), 1454. doi: 10.3390/foods10071454

- Long, N. H. B. S., Gál, R., & Buňka, F. (2011). Use of phosphates in meat products. *African Journal of Biotechnology, 10*(86), 19874-19882.

- López-López, I., Cofrades, S., Ruiz-Capillas, C., & Jiménez-Colmenero, F. (2009). Design and nutritional properties of potential functional frankfurters based on lipid formulation, added seaweed and low salt content. *Meat science, 83*(2), 255-262. doi: 10.1016/j.meatsci.2009.05.014

- Lowder, A. C., Goad, C. L., Lou, X., Morgan, J. B., & Mireles DeWitt, C. A. (2011). Evaluation of a dehydrated beef protein to replace sodium-based phosphates in injected beef strip loins. *Meat Science, 89*(4), 491-499. doi: 10.1016/j.meatsci.2011.05.013

- Lu, X., Zhang, H., Li, Y., & Huang, Q. (2018). Fabrication of milled cellulose particles-stabilized Pickering emulsions. *Food Hydrocolloids, 77*, 427-435.

- Lu, Z., Ye, F., Zhou, G., Gao, R., Qin, D., & Zhao, G. (2020). Micronized apple pomace as a novel emulsifier for food O/W Pickering emulsion. *Food chemistry, 330*, 127325-127325. doi: 10.1016/j.foodchem.2020.127325

- Luo, X., Wang, Q., Zheng, B., Lin, L., Chen, B., Zheng, Y., & Xiao, J. (2017). Hydration properties and binding capacities of dietary fibers from bamboo shoot shell and its hypolipidemic effects in mice. *Food and chemical toxicology, 109*(Pt 2), 1003-1009. doi: 10.1016/j.fct.2017.02.029

- MacDonald, P. L., & Gardner, R. C. (2000). Type I Error Rate Comparisons of Post Hoc Procedures for I j Chi-Square Tables. *Educational and psychological measurement, 60*(5), 735-754. doi: 10.1177/00131640021970871

- Machado, S., Costa, A. S. G., Pimentel B, F., Oliveira, M. B. P. P., & Alves, R. C. (2020). A study on the protein fraction of coffee silverskin: Protein/non-protein nitrogen and free and total amino acid profiles. *Food chemistry, 326*, 126940-126940. doi: 10.1016/j.foodchem.2020.126940

- Madane, P., Das, A. K., Nanda, P. K., Bandyopadhyay, S., Jagtap, P., Shewalkar, A., & Maity, B. (2019). Dragon fruit (Hylocereus undatus) peel as antioxidant dietary fibre on quality and lipid oxidation of chicken nuggets. *Journal of food science and technology, 57*(4), 1449-1461. doi: 10.1007/s13197-019-04180-z

- Maksimenko, A., Kikuchi, R., Tsutsuura, S., & Nishiumi, T. (2019). Effect of high hydrostatic pressure and reducing sodium chloride and phosphate on

physicochemical properties of beef gels. *High pressure research, 39*(2), 385-397. doi: 10.1080/08957959.2019.1586895

- Maraschiello, C., Sárraga, C., & García Regueiro, J. A. (1999). Glutathione Peroxidase Activity, TBARS, and α-Tocopherol in Meat from Chickens Fed Different Diets. *Journal of agricultural and food chemistry, 47*(3), 867-872. doi: 10.1021/jf980824o
- Martuscelli, M., Esposito, L., & Mastrocola, D. (2021). The Role of Coffee Silver Skin against Oxidative Phenomena in Newly Formulated Chicken Meat Burgers after Cooking. *Foods, 10*(8), 1833. doi: 10.3390/foods10081833
- Martuscelli, M., Esposito, L., Di Mattia, C., Ricci, A., & Mastrocola, D. (2021). Characterization of Coffee Silver Skin as Potential Food-Safe Ingredient. *Foods, 10*(6), 1367. doi: 10.3390/foods10061367
- McClemens, D. J., Friberg, S., Larsson, K., & Sjoblom, J., (2016). In: Food Emulsions, CRC press, Boca Raton, FL. doi:10.1201/9780203913222
- McDonnell, C. K., Allen, P., Duane, G., Morin, C., Casey, E., & Lyng, J. G. (2018). One-directional modelling to assess the mechanistic actions of power ultrasound on NaCl diffusion in pork. *Ultrasonics sonochemistry, 40*(Pt A), 206-212. doi: 10.1016/j.ultsonch.2017.06.025
- McDonnell, C. K., Allen, P., Duggan, E., Arimi, J. M., Casey, E., Duane, G., & Lyng, J. G. (2013). The effect of salt and fibre direction on water dynamics, distribution and mobility in pork muscle: A low field NMR study. *Meat Science, 95*(1), 51-58. doi: 10.1016/j.meatsci.2013.04.012
- McDonnell, C. K., Lyng, J. G., Arimi, J. M., & Allen, P. (2014). The acceleration of pork curing by power ultrasound: A pilot-scale production. *Innovative food science & emerging technologies, 26*, 191-198. doi: 10.1016/j.ifset.2014.05.004
- McKenna, B., & Lyng, J. (2003). Introduction to food rheology and its measurement. *Texture in food, 1*, 130-160.
- Menozzi, D., Halawany-Darson, R., Mora, C., & Giraud, G. (2015). Motives towards traceable food choice: A comparison between French and Italian consumers. *Food control, 49*, 40-48. doi: 10.1016/j.foodcont.2013.09.006
- Menozzi, D., Sogari, G., Veneziani, M., Simoni, E., & Mora, C. (2017). Eating novel foods: An application of the Theory of Planned Behaviour to predict the consumption of an insect-based product. *Food quality and preference, 59*, 27-34. doi: 10.1016/j.foodqual.2017.02.001
- Moczkowska, M., Karp, S., Niu, Y., & Kurek, M. A. (2019). Enzymatic, enzymatic-ultrasonic and alkaline extraction of soluble dietary fibre from flaxseed – A physicochemical approach. *Food hydrocolloids, 90*, 105-112. doi: 10.1016/j.foodhyd.2018.12.018
- Moiseev, I. V., & Cornforth, D. P. (1997). Sodium hydroxide and sodium tripolyphosphate effects on bind strength and sensory characteristics of

restructured beef rolls. *Meat Science, 45*(1), 53-60. doi: 10.1016/S0309-1740(96)00093-9

- Mokrzycki, W., & Tatol, M. (2011). Colour differenceΔ E-A survey. *Mach. Graph. Vis, 20*(4), 383-411.
- Moreira, M. N. B., da Veiga, C. P., da Veiga, C. R. P., Reis, G. G., & Pascuci, L. M. (2022). Reducing meat consumption: Insights from a bibliometric analysis and future scopes. *Future Foods, 5*, 100120.
- Mørk, T., Lähteenmäki, L., & Grunert, K. G. (2019). Determinants of intention to reduce salt intake and willingness to purchase salt-reduced food products: Evidence from a web survey. *Appetite, 139*, 110-118. doi: 10.1016/j.appet.2019.04.018
- Morrissey, P. A., Sheehy, P. J. A., Galvin, K., Kerry, J. P., & Buckley, D. J. (1998). Lipid stability in meat and meat products. *Meat Science*, *49*, S73-S86.
- Mudalal, S., Petracci, M., Tappi, S., Rocculi, P., & Cavani, C. (2014). Comparison between the quality traits of phosphate and bicarbonate-marinated chicken breast fillets cooked under different heat treatments. *Food and Nutrition Sciences, 2014*.
- Mulet, A., Cárcel, J. A., Sanjuán, N., & Bon, J. (2003). New Food Drying Technologies - Use of Ultrasound. *Food science and technology international, 9*(3), 215-221. doi: 10.1177/1082013203034641
- Mullen, A. M., Álvarez, C., Pojić, M., Hadnadev, T. D., & Papageorgiou, M. (2015). Classification and target compounds *Food waste recovery* (pp. 25-57): Elsevier.
- Muntean, M.-V., Marian, O., Barbieru, V., Cătunescu, G. M., Ranta, O., Drocas, I., & Terhes, S. (2016). High pressure processing in food industry–characteristics and applications. *Agriculture and Agricultural Science Procedia, 10*, 377-383.
- Murthy, P. S., & Naidu, M. M. (2010). Recovery of Phenolic Antioxidants and Functional Compounds from Coffee Industry By-Products. *Food and bioprocess technology, 5*(3), 897-903. doi: 10.1007/s11947-010-0363-z
- Navarro-González, I., García-Valverde, V., García-Alonso, J., & Periago, M. J. (2011). Chemical profile, functional and antioxidant properties of tomato peel fiber. *Food research international, 44*(5), 1528-1535. doi: 10.1016/j.foodres.2011.04.005
- Nowak, D., & Jakubczyk, E. (2020). The freeze-drying of foods—The characteristic of the process course and the effect of its parameters on the physical properties of food materials. *Foods, 9*(10), 1488.
- Nunes, L. J., Matias, J. C. D. O., & Catalao, J. P. D. S. (2017). *Torrefaction of biomass for energy applications: from fundamentals to industrial scale*: Academic Press.

- O'Donnell, C. P., Tiwari, B. K., Bourke, P., & Cullen, P. J. (2010). Effect of ultrasonic processing on food enzymes of industrial importance. *Trends in food science & technology, 21*(7), 358-367. doi: 10.1016/j.tifs.2010.04.007
- O'Farrell, M. (2011). Online quality assessment of processed meats *Processed Meats* (pp. 546-566): Elsevier.
- O'Flynn, C. C., Cruz-Romero, M. C., Troy, D. J., Mullen, A. M., & Kerry, J. P. (2014). The application of high-pressure treatment in the reduction of phosphate levels in breakfast sausages. *Meat Science, 96*(1), 633-639. doi: 10.1016/j.meatsci.2013.08.028
- O'Flynn, C. C., Cruz-Romero, M. C., Troy, D., Mullen, A. M., & Kerry, J. P. (2014). The application of high-pressure treatment in the reduction of salt levels in reduced-phosphate breakfast sausages. *Meat Science, 96*(3), 1266-1274. doi: 10.1016/j.meatsci.2013.11.010
- Ojha, K. S., Keenan, D. F., Bright, A., Kerry, J. P., & Tiwari, B. K. (2016). Ultrasound assisted diffusion of sodium salt replacer and effect on physicochemical properties of pork meat. *International journal of food science & technology, 51*(1), 37-45. doi: 10.1111/ijfs.13001
- Omana, D. A., Plastow, G., & Betti, M. (2011). Effect of different ingredients on color and oxidative characteristics of high pressure processed chicken breast meat with special emphasis on use of β-glucan as a partial salt replacer. *Innovative food science & emerging technologies, 12*(3), 244-254. doi: 10.1016/j.ifset.2011.04.007
- O'Neill, C. M., Cruz-Romero, M. C., Duffy, G., & Kerry, J. P. (2018). The application of response surface methodology for the development of sensory accepted low-salt cooked ham using high pressure processing and a mix of organic acids. Innovative food science & emerging technologies, 45, 401-411. doi:10.1016/j.ifset.2017.12.009
- O'Neill, C. M., Cruz-Romero, M. C., Duffy, G., & Kerry, J. P. (2019). The application of response surface methodology for development of sensory acceptable, low-salt, shelf-stable frankfurters using high-pressure processing and a mix of organic acids. European Food Research and Technology, 245(6), 1277-1291. doi:10.1007/s00217-019-03243-x
- OSullivan, C. M., Lynch, A.-M., Lynch, P. B., Buckley, D. J., & Kerry, J. P. (2004). Use of antioxidants in chicken nuggets manufactured with and without the use of salt and/or sodium tripolyphosphate: Effects on product quality and shelf-life stability. *International Journal of Poultry Science*.
- Öztürk, B., & Serdaroğlu, M. (2017). *Effects of inulin and sodium carbonate in phosphate-free restructured poultry steaks.* Paper presented at the IOP Conference Series: Earth and Environmental Science.
- Ozuna, C., Paniagua-Martínez, I., Castaño-Tostado, E., Ozimek, L., & Amaya-Llano, S. L. (2015). Innovative applications of high-intensity ultrasound in the

development of functional food ingredients: Production of protein hydrolysates and bioactive peptides. *Food research international, 77*, 685-696. doi: 10.1016/j.foodres.2015.10.015

- Ozuna, C., Puig, A., García-Pérez, J. V., Mulet, A., & Cárcel, J. A. (2013). Influence of high intensity ultrasound application on mass transport, microstructure and textural properties of pork meat (Longissimus dorsi) brined at different NaCl concentrations. *Journal of food engineering, 119*(1), 84-93. doi: 10.1016/j.jfoodeng.2013.05.016

- Paglarini, C. d. S., Furtado, G. d. F., Biachi, J. P., Vidal, V. A. S., Martini, S., Forte, M. B. S., Cunha, R. L., & Pollonio, M. A. R. (2018). Functional emulsion gels with potential application in meat products. *Journal of food engineering, 222*, 29-37. doi: 10.1016/j.jfoodeng.2017.10.026

- Paglarini, C. d. S., Martini, S., & Pollonio, M. A. R. (2019). Using emulsion gels made with sonicated soy protein isolate dispersions to replace fat in frankfurters. *Food science & technology, 99*, 453-459. doi: 10.1016/j.lwt.2018.10.005

- Parsons, A. N., VanOverbeke, D. L., Goad, C. L., & DeWitt, C. A. M. (2011). Retail Display Evaluation of Steaks from Select Beef Strip Loins Injected with a Brine Containing 1% Ammonium Hydroxide. Part 2: Cook Yield, Tenderness, and Sensory Attributes. *Journal of food science, 76*(1), S84-S88. doi: 10.1111/j.1750-3841.2010.01903.x

- Parsons, A. N., VanOverbeke, D. L., Goad, C. L., & Mireles DeWitt, C. A. (2011). Retail Display Evaluation of Steaks from Select Beef Strip Loins Injected with a Brine Containing 1% Ammonium Hydroxide. Part 1: Fluid Loss, Oxidation, Color, and Microbial Plate Counts. *Journal of food science, 76*(1), S63-S71. doi: 10.1111/j.1750-3841.2010.01892.x

- Patist, A., & Bates, D. (2011). Industrial applications of high power ultrasonics *Ultrasound technologies for food and bioprocessing* (pp. 599-616): Springer.

- Pearce, K. L., Rosenvold, K., Andersen, H. J., & Hopkins, D. L. (2011). Water distribution and mobility in meat during the conversion of muscle to meat and ageing and the impacts on fresh meat quality attributes — A review. *Meat Science, 89*(2), 111-124. doi: 10.1016/j.meatsci.2011.04.007

- Pérez-Andrés, J. M., de Alba, M., Harrison, S. M., Brunton, N. P., Cullen, P., & Tiwari, B. K. (2020). Effects of cold atmospheric plasma on mackerel lipid and protein oxidation during storage. *Lwt, 118*, 108697.

- Pérez-Montes, A., Rangel-Vargas, E., Lorenzo, J. M., Romero, L., & Santos, E. M. (2021). Edible mushrooms as a novel trend in the development of healthier meat products. *Current opinion in food science, 37*, 118-124. doi: 10.1016/j.cofs.2020.10.004

- Petersson, K., Godard, O., Eliasson, A.-C., & Tornberg, E. (2014). The effects of cereal additives in low-fat sausages and meatballs. Part 2: Rye bran, oat bran and barley fibre. *Meat Science, 96*(1), 503-508.

- Petracci, M., Bianchi, M., Mudalal, S., & Cavani, C. (2013). Functional ingredients for poultry meat products. *Trends in food science & technology, 33*(1), 27-39.
- Petracci, M., Laghi, L., Rocculi, P., Rimini, S., Panarese, V., Cremonini, M., & Cavani, C. (2012). The use of sodium bicarbonate for marination of broiler breast meat. *Poultry Science, 91*(2), 526-534.
- Pietrasik, Z., & Janz, J. A. M. (2010). Utilization of pea flour, starch-rich and fiber-rich fractions in low fat bologna. *Food research international, 43*(2), 602-608. doi: 10.1016/j.foodres.2009.07.017
- Pinton, M. B., Correa, L. P., Facchi, M. M. X., Heck, R. T., Leães, Y. S. V., Cichoski, A. J., Lorenzo, J. M., dos Santos, M., Pollonio, M. A. R., & Campagnol, P. C. B. (2019). Ultrasound: A new approach to reduce phosphate content of meat emulsions. *Meat Science, 152*, 88-95. doi: 10.1016/j.meatsci.2019.02.010
- Pinton, M. B., dos Santos, B. A., Lorenzo, J. M., Cichoski, A. J., Boeira, C. P., & Campagnol, P. C. B. (2021). Green technologies as a strategy to reduce NaCl and phosphate in meat products: an overview. *Current opinion in food science, 40*, 1-5. doi: 10.1016/j.cofs.2020.03.011
- Pourfarzad, A., Mahdavian-Mehr, H., & Sedaghat, N. (2013). Coffee silverskin as a source of dietary fiber in bread-making: Optimization of chemical treatment using response surface methodology. *Food science & technology, 50*(2), 599-606. doi: 10.1016/j.lwt.2012.08.001
- Powell, M. (2017). Physical and chemical effects of citrus fiber as a natural alternative to sodium tripolyphosphate in uncured all-pork bologna and oven-roasted turkey breast (Master's thesis). *Iowa State University. Iowa, United States*.
- Prabhu, G., & Husak, R. (2014). Use of sodium carbonate and native potato starch blends as a phosphate replacer in natural enhanced pork loins. *Meat Science, 1*(96), 454-455.
- Prati, G., Pietrantoni, L., & Zani, B. (2012). The prediction of intention to consume genetically modified food: Test of an integrated psychosocial model. *Food quality and preference, 25*(2), 163-170. doi: 10.1016/j.foodqual.2012.02.011
- Priyadarshini, A., Rajauria, G., O'Donnell, C. P., & Tiwari, B. K. (2019). Emerging food processing technologies and factors impacting their industrial adoption. *Critical Reviews in Food Science and Nutrition, 59*(19), 3082-3101. doi: 10.1080/10408398.2018.1483890
- Puolanne, E. J., Ruusunen, M. H., & Vainionpää, J. I. (2001). Combined effects of NaCl and raw meat pH on water-holding in cooked sausage with and without added phosphate. *Meat Science, 58*(1), 1-7. doi: 10.1016/S0309-1740(00)001236
- Puolanne, E., & Halonen, M. (2010). Theoretical aspects of water-holding in meat. *Meat Science, 86*(1), 151-165. doi: 10.1016/j.meatsci.2010.04.038

- Qin, W., & Brown, J. L. (2008). Factors explaining male/female differences in attitudes and purchase intention toward genetically engineered salmon. *Journal of consumer behaviour, 7*(2), 127-145. doi: 10.1002/cb.242
- Raghavendra, S. N., Rastogi, N. K., Raghavarao, K. S. M. S., & Tharanathan, R. N. (2004). Dietary fiber from coconut residue: effects of different treatments and particle size on the hydration properties. *European food research & technology, 218*(6), 563-567. doi: 10.1007/s00217-004-0889-2
- Rastogi, N. K. (2011). Opportunities and Challenges in Application of Ultrasound in Food Processing. *Critical Reviews in Food Science and Nutrition, 51*(8), 705-722. doi: 10.1080/10408391003770583
- Rather, S. A., Akhter, R., Masoodi, F., Gani, A., & Wani, S. (2015). Utilization of apple pomace powder as a fat replacer in goshtaba: a traditional meat product of Jammu and Kashmir, India. *Journal of Food measurement and Characterization, 9*(3), 389-399.
- Regulation (EC) No 1333/2008 of the European Parliament and of the council of 16 December 2008 on food additives. In: Official Journal of the European Union.
- Regulation (EC) No 853/2004 of European Parliament and of the Council of 29 April laying down specific hygiene rules for the hygiene of foodstuffs., (2004).
- Ren, F., Feng, Y., Zhang, H., & Wang, J. (2021). Effects of modification methods on microstructural and physicochemical characteristics of defatted rice bran dietary fiber. *Food science & technology, 151*, 112161. doi: 10.1016/j.lwt.2021.112161
- Resconi, V. C., Keenan, D. F., Barahona, M., Guerrero, L., Kerry, J. P., & Hamill, R. M. (2016). Rice starch and fructo-oligosaccharides as substitutes for phosphate and dextrose in whole muscle cooked hams: Sensory analysis and consumer preferences. *Food science & technology, 66*, 284-292. doi: 10.1016/j.lwt.2015.10.048
- Resconi, V. C., Keenan, D. F., García, E., Allen, P., Kerry, J. P., & Hamill, R. M. (2016). The effects of potato and rice starch as substitutes for phosphate in and degree of comminution on the technological, instrumental and sensory characteristics of restructured ham. *Meat Science, 121*, 127-134. doi: 10.1016/j.meatsci.2016.05.017
- Resconi, V. C., Keenan, D. F., Gough, S., Doran, L., Allen, P., Kerry, J. P., & Hamill, R. M. (2015). Response surface methodology analysis of rice starch and fructo-oligosaccharides as substitutes for phosphate and dextrose in whole muscle cooked hams. *Food science & technology, 64*(2), 946-958. doi: 10.1016/j.lwt.2015.06.053
- Resendiz-Vazquez, J. A., Ulloa, J. A., Urías-Silvas, J. E., Bautista-Rosales, P. U., Ramírez-Ramírez, J. C., Rosas-Ulloa, P., & González-Torres, L. (2017). Effect of high-intensity ultrasound on the technofunctional properties and structure of jackfruit (Artocarpus heterophyllus) seed protein isolate. *Ultrasonics sonochemistry, 37*, 436-444. doi: 10.1016/j.ultsonch.2017.01.042

- Rigdon, M., Hung, Y.-C., & Stelzleni, A. M. (2017). Evaluation of alkaline electrolyzed water to replace traditional phosphate enhancement solutions: Effects on water holding capacity, tenderness, and sensory characteristics. *Meat Science, 123*, 211-218. doi: 10.1016/j.meatsci.2016.10.007

- Ritchie, D., Van den Broucke, S., & Van Hal, G. (2021). The health belief model and theory of planned behavior applied to mammography screening: A systematic review and meta-analysis. *Public health nursing (Boston, Mass.), 38*(3), 482-492. doi: 10.1111/phn.12842

- Ritz, E., Hahn, K., Ketteler, M., Kuhlmann, M. K., & Mann, J. (2012). Phosphate additives in food--a health risk. *Deutsches Ärzteblatt international, 109*(4), 49-55. doi: 10.3238/arztebl.2012.0049

- Rodriguez Furlán, L. T., Padilla, A. P., & Campderrós, M. E. (2014). Development of reduced fat minced meats using inulin and bovine plasma proteins as fat replacers. *Meat Science, 96*(2), 762-768. doi: 10.1016/j.meatsci.2013.09.015

- Rohrmann, S., & Linseisen, J. (2016). Processed meat: the real villain? *Proceedings of the Nutrition Society, 75*(3), 233-241. doi: 10.1017/S0029665115004255

- Sah, B. N. P., Vasiljevic, T., McKechnie, S., & Donkor, O. N. (2016). Physicochemical, textural and rheological properties of probiotic yogurt fortified with fibre-rich pineapple peel powder during refrigerated storage. *Food science & technology, 65*, 978-986. doi: 10.1016/j.lwt.2015.09.027

- Salgado, P. R., Fernández, G. B., Drago, S. R., & Mauri, A. N. (2011). Addition of bovine plasma hydrolysates improves the antioxidant properties of soybean and sunflower protein-based films. *Food hydrocolloids, 25*(6), 1433-1440. doi: 10.1016/j.foodhyd.2011.02.003

- Sampedro, F., McAloon, A., Yee, W., Fan, X., & Geveke, D. J. (2014). Cost Analysis and Environmental Impact of Pulsed Electric Fields and High Pressure Processing in Comparison with Thermal Pasteurization. *Food and Bioprocess Technology, 7*(7), 1928-1937. doi: 10.1007/s11947-014-1298-6

- Sanjeewa, W. G. T., Wanasundara, J. P. D., Pietrasik, Z., & Shand, P. J. (2010). Characterization of chickpea (Cicer arietinum L.) flours and application in low-fat pork bologna as a model system. *Food research international, 43*(2), 617-626. doi: 10.1016/j.foodres.2009.07.024

- Santos, M. d., Ozaki, M. M., Ribeiro, W. O., Paglarini, C. d. S., Vidal, V. A. S., Campagnol, P. C. B., & Pollonio, M. A. R. (2020). Emulsion gels based on pork skin and dietary fibers as animal fat replacers in meat emulsions: An adding value strategy to byproducts. *Food science & technology, 120*, 108895. doi: 10.1016/j.lwt.2019.108895

- Saratale, G. D., Bhosale, R., Shobana, S., Banu, J. R., Pugazhendhi, A., Mahmoud, E., Sirohi, R., Bhatia, K. S., Atabani, A. E., Mulone, V., Yoon, J-J., Shin, S. H., & Kumar, G. (2020). A review on valorization of spent coffee grounds (SCG) towards

biopolymers and biocatalysts production. *Bioresource technology, 314*, 123800-123800. doi: 10.1016/j.biortech.2020.123800

- Sarıçoban, C., Özalp, B., Yılmaz, M. T., Özen, G., Karakaya, M., & Akbulut, M. (2008). Characteristics of meat emulsion systems as influenced by different levels of lemon albedo. *Meat Science, 80*(3), 599-606. doi: https://doi.org/10.1016/j.meatsci.2008.02.008

- Schwartz, W. C., & Mandigo, R. W. (1976). Effect of salt, sodium tripolyphosphate and storage on restructured pork. *Journal of food science, 41*(6), 1266-1269. doi: 10.1111/j.1365-2621.1976.tb01148.x

- Sen, A., Naveena, B., Muthukumar, M., Babji, Y., & Murthy, T. (2005). Effect of chilling, polyphosphate and bicarbonate on quality characteristics of broiler breast meat. *British poultry science, 46*(4), 451-456.

- Shan, L. C., De Brún, A., Henchion, M., Li, C., Murrin, C., Wall, P. G., & Monahan, F. J. (2017). Consumer evaluations of processed meat products reformulated to be healthier – A conjoint analysis study. *Meat Science, 131*, 82-89. doi: 10.1016/j.meatsci.2017.04.239

- Shan, L. C., Regan, A., Monahan, F. J., Li, C., Murrin, C., Lalor, F., Wall, P. G., McConnon, A. (2016). Consumer views on "healthier" processed meat. *British food journal (1966), 118*(7), 1712-1730. doi: 10.1108/BFJ-11-2015-0447

- Sharpe, D. (2015). Chi-Square Test is Statistically Significant: Now What? *Practical assessment, research & evaluation, 20*, 8.

- Shults, G., Russell, D., & Wierbicki, E. (1972). Effect of condensed phosphates on pH, swelling and water-holding capacity of beef. *Journal of Food Science, 37*(6), 860-864.

- Sievert, K., Lawrence, M., Parker, C., Russell, C. A., & Baker, P. (2022). Who has a beef with reducing red and processed meat consumption? A media framing analysis. *Public health nutrition, 25*(3), 578-590. doi: 10.1017/S1368980021004092

- Simuang, J., Chiewchan, N., & Tansakul, A. (2004). Effects of fat content and temperature on the apparent viscosity of coconut milk. *Journal of food engineering, 64*(2), 193-197. doi: 10.1016/j.jfoodeng.2003.09.032

- Siró, I., Vén, C., Balla, C., Jónás, G., Zeke, I., & Friedrich, L. (2009). Application of an ultrasonic assisted curing technique for improving the diffusion of sodium chloride in porcine meat. *Journal of food engineering, 91*(2), 353-362. doi: 10.1016/j.jfoodeng.2008.09.015

- Soladoye, O. P., Pietrasik, Z., Hrynets, Y., & Betti, M. (2021). The effect of glucosamine and glucosamine caramel on quality and consumer acceptability of regular and reduced salt breakfast sausages. *Meat Science, 172*, 108310-108310. doi: 10.1016/j.meatsci.2020.108310

- Sorenson, D., Henchion, M., Marcos, B., Ward, P., Mullen, A. M., & Allen, P. (2011). Consumer acceptance of high pressure processed beef-based chilled ready meals: The mediating role of food-related lifestyle factors. *Meat Science, 87*(1), 81-87. doi: 10.1016/j.meatsci.2010.09.006

- Sousa, S. C., Fragoso, S. P., Penna, C. R., Arcanjo, N. M., Silva, F. A., Ferreira, V. C., Barreto, M. D., & Araújo, Í. B. (2017). Quality parameters of frankfurter-type sausages with partial replacement of fat by hydrolyzed collagen. *LWT-Food Science and Technology, 76*, 320-325.

- Sujka, M., & Jamroz, J. (2013). Ultrasound-treated starch: SEM and TEM imaging, and functional behaviour. *Food hydrocolloids, 31*(2), 413-419. doi: 10.1016/j.foodhyd.2012.11.027

- Sun, X. D., & Arntfield, S. D. (2012). Molecular forces involved in heat-induced pea protein gelation: Effects of various reagents on the rheological properties of salt-extracted pea protein gels. *Food hydrocolloids, 28*(2), 325-332. doi: 10.1016/j.foodhyd.2011.12.014

- Sutton, S. (1998). Predicting and Explaining Intentions and Behavior: How Well Are We Doing? *Journal of applied social psychology, 28*(15), 1317-1338. doi: 10.1111/j.1559-1816.1998.tb01679.x

- Takeda, E., Yamamoto, H., Yamanaka-Okumura, H., & Taketani, Y. (2014). Increasing dietary phosphorus intake from food additives: potential for negative impact on bone health. *Advances in nutrition (Bethesda, Md.), 5*(1), 92-97. doi: 10.3945/an.113.004002

- Talukder, S. (2015). Effect of dietary fiber on properties and acceptance of meat products: a review. *Critical reviews in food science and nutrition, 55*(7), 1005-1011.

- Tamm, A., Bolumar, T., Bajovic, B., & Toepfl, S. (2016). Salt (NaCl) reduction in cooked ham by a combined approach of high pressure treatment and the salt replacer KCl. *Innovative food science & emerging technologies, 36*, 294-302. doi: 10.1016/j.ifset.2016.07.010

- Teixeira, A., & Rodrigues, S. (2021). Consumer perceptions towards healthier meat products. *Current opinion in food science, 38*, 147-154. doi: 10.1016/j.cofs.2020.12.004

- Téllez-Morales, J. A., Hernández-Santo, B., & Rodríguez-Miranda, J. (2020). Effect of ultrasound on the techno-functional properties of food components/ingredients: A review. *Ultrasonics sonochemistry, 61*, 104787-104787. doi: 10.1016/j.ultsonch.2019.104787

- Tiwari, U., & Cummins, E. (2011). Functional and physicochemical properties of legume fibers.

- Toepfl, S., Heinz, V., & Knorr, D. (2006). *Pulsed electric fields (PEF) processing of meat.* Paper presented at the 13th World Congress of Food Science & Technology 2006.

- Tomasevic, I., Djekic, I., Font-i-Furnols, M., Terjung, N., & Lorenzo, J. M. (2021). Recent advances in meat color research. *Current opinion in food science, 41*, 81-87. doi: 10.1016/j.cofs.2021.02.012
- Troy, D. J., Ojha, K. S., Kerry, J. P., & Tiwari, B. K. (2016). Sustainable and consumer-friendly emerging technologies for application within the meat industry: An overview. *Meat Science, 120*, 2-9.
- Tseng, T.-F., Liu, D.-C., & Chen, M.-T. (2000). Evaluation of transglutaminase on the quality of low-salt chicken meat-balls. *Meat Science, 55*(4), 427-431. doi: 10.1016/S0309-1740(99)00172-2
- Ünver, A. (2016). Applications of ultrasound in food processing. *Green Chemical and Technological Letters, 2*(3), 121-126.
- Vervloet, M. G., & van Ballegooijen, A. J. (2018). Prevention and treatment of hyperphosphatemia in chronic kidney disease. *Kidney international, 93*(5), 1060-1072. doi: 10.1016/j.kint.2017.11.036
- Vimercati, W. C., Araújo, C. d. S., Macedo, L. L., Correa, J. L. G., & Pimenta, C. J. (2022). Encapsulation of coffee silverskin extracts by foam mat drying and comparison with powders obtained by spray drying and freeze-drying. *Journal of Food Science*.
- Wachirasiri, K., Wanlapa, S., Uttapap, D., Puttanlek, C., & Rungsardthong, V. (2017). Changing in processing yield and physical properties of frozen white shrimp (Penaeus vannamei) treated with lysine and sodium bicarbonate. *International Journal of Food Science & Technology, 52*(3), 763-771.
- Wang, P., Xu, X.-l., & Zhou, G.-h. (2009). Effects of Meat and Phosphate Level on Water-Holding Capacity and Texture of Emulsion-Type Sausage during Storage. *Agricultural sciences in China, 8*(12), 1475-1481. doi: 10.1016/S1671-2927(08)60361
- Wang, Y., Sun, P., Li, H., Adhikari, B. P., & Li, D. (2018). Rheological Behavior of Tomato Fiber Suspensions Produced by High Shear and High Pressure Homogenization and Their Application in Tomato Products. *International journal of analytical chemistry, 2018*, 5081938-5081912. doi: 10.1155/2018/5081938
- Warner, R. D. (2017). The eating quality of meat—IV Water-holding capacity and juiciness *Lawrie´ s meat science* (pp. 419-459): Elsevier.
- Warner, R. D., McDonnell, C. K., Bekhit, A. E. D., Claus, J., Vaskoska, R., Sikes, A., Dunshea, F. R., & Ha, M. (2017). Systematic review of emerging and innovative technologies for meat tenderisation. *Meat Science, 132*, 72-89.
- Whitnall, T., & Pitts, N. (2019). Global trends in meat consumption. Agricultural Commodities, 9(1), 96-99.
- Winger, R. J., Uribarri, J., & Lloyd, L. (2012). Phosphorus-containing food additives: An insidious danger for people with chronic kidney disease. *Trends in food science & technology, 24*(2), 92-102. doi: 10.1016/j.tifs.2011.11.001

- Wolstenholme, E., Carfora, V., Catellani, P., Poortinga, W., & Whitmarsh, L. (2021). Explaining intention to reduce red and processed meat in the UK and Italy using the theory of planned behaviour, meat-eater identity, and the Transtheoretical model. *Appetite, 166*, 105467-105467. doi: 10.1016/j.appet.2021.105467

- Xue, S., Wang, H., Yang, H., Yu, X., Bai, Y., Tendu, A. A., Xu, X., Ma, H., & Zhou, G. (2017). Effects of high-pressure treatments on water characteristics and juiciness of rabbit meat sausages: Role of microstructure and chemical interactions. *Innovative food science & emerging technologies, 41*, 150-159. doi: 10.1016/j.ifset.2017.03.006

- Yadav, S., Malik, A., Pathera, A., Islam, R. U., & Sharma, D. (2016). Development of dietary fibre enriched chicken sausages by incorporating corn bran, dried apple pomace and dried tomato pomace. *Nutrition and food science, 46*(1), 16-29. doi: 10.1108/NFS-05-2015-0049

- Yang, H., Han, M., Wang, X., Han, Y., Wu, J., Xu, X., & Zhou, G. (2015). Effect of high pressure on cooking losses and functional properties of reduced-fat and reduced-salt pork sausage emulsions. *Innovative food science & emerging technologies, 29*, 125-133. doi: 10.1016/j.ifset.2015.02.013

- Yang, H., Tao, F., Cao, G., Han, M., Xu, X., Zhou, G., & Shen, Q. (2021). Stability improvement of reduced-fat reduced-salt meat batter through modulation of secondary and tertiary protein structures by means of high pressure processing. *Meat Science, 176*, 108439-108439. doi: 10.1016/j.meatsci.2021.108439

- Yegin, S., Kopec, A., Kitts, D. D., & Zawistowski, J. (2020). Dietary fiber: A functional food ingredient with physiological benefits *Dietary Sugar, Salt and Fat in Human Health* (pp. 531-555): Elsevier.

- Yolmeh, M., Khomeiri, M., & Ahmadi, Z. (2017). Application of mixture design to introduce an optimum cell-free supernatant of multiple-strain mixture (MSM) for Lactobacillus against food-borne pathogens. *Food science & technology, 83*, 298-304. doi: 10.1016/j.lwt.2017.05.035

- Younes, M., Aquilina, G., Castle, L., Engel, K. H., Fowler, P., Frutos Fernandez, M. J., Fürst, P., Gürtler, R., Husøy, T., Mennes, W., Moldeus, P., Oskarsson, A., Shah, R., Wallkens-Berendsen, I., Wölfle, D., Aggett, P., Cupisti, A., Fortes, C., Kuhnle, G., Lillegaard, I T., Scotter, M., Giarola, A., Rincon, A., Tard, A., Gundert-Remy, U., & Sveriges, l. (2019). Re-evaluation of phosphoric acid–phosphates – di-, tri- and polyphosphates (E 338–341, E 343, E 450–452) as food additives and the safety of proposed extension of use. *EFSA journal, 17*(6), e05674-n/a. doi: 10.2903/j.efsa.2019.5674

- Younis, K., & Ahmad, S. (2015). Waste utilization of apple pomace as a source of functional ingredient in buffalo meat sausage. *Cogent food & agriculture, 1*(1), 1119397. doi: 10.1080/23311932.2015.1119397

- Younis, K., & Ahmad, S. (2018). Quality evaluation of buffalo meat patties incorporated with apple pomace powder. *Buffalo Bulletin, 37*(3), 389-401.

- Younis, K., Ahmad, S., & Malik, M. A. (2021). Mosambi peel powder incorporation in meat products: Effect on physicochemical properties and shelf life stability. *Applied Food Research, 1*(2), 100015.

- Younis, K., Yousuf, O., Qadri, O. S., Jahan, K., Osama, K., & Islam, R. U. (2022). Incorporation of soluble dietary fiber in comminuted meat products: Special emphasis on changes in textural properties. *Bioactive carbohydrates and dietary fibre, 27*, 100288. doi: 10.1016/j.bcdf.2021.100288

- Youssef, M. K., & Barbut, S. (2011). Effects of two types of soy protein isolates, native and preheated whey protein isolates on emulsified meat batters prepared at different protein levels. *Meat Science, 87*(1), 54-60. doi: 10.1016/j.meatsci.2010.09.002

- Yum, H.-W., Seo, J.-K., Jeong, J.-Y., Kim, G.-D., Rahman, M. S., & Yang, H.-S. (2018). The quality improvement of emulsion-type pork sausages formulated by substituting pork back fat with rice bran oil. *Korean Journal for Food Science of Animal Resources, 38*(1), 123.

- Zhang, C., Liu, H., Xia, X., Sun, F., & Kong, B. (2021). Effect of ultrasound-assisted immersion thawing on emulsifying and gelling properties of chicken myofibrillar protein. *Food science & technology, 142*, 111016. doi: 10.1016/j.lwt.2021.111016

- Zhang, W., Zeng, G., Pan, Y., Chen, W., Huang, W., Chen, H., & Li, Y. (2017). Properties of soluble dietary fiber-polysaccharide from papaya peel obtained through alkaline or ultrasound-assisted alkaline extraction. *Carbohydrate polymers, 172*, 102-112. doi: 10.1016/j.carbpol.2017.05.030

- Zhang, Y., Holman, B. W. B., Ponnampalam, E. N., Kerr, M. G., Bailes, K. L., Kilgannon, A. K., Collins, D., & Hopkins, D. L. (2019). Understanding beef flavour and overall liking traits using two different methods for determination of thiobarbituric acid reactive substance (TBARS). *Meat Science, 149*, 114-119. doi: 10.1016/j.meatsci.2018.11.018

- Zheng, H.-b., Han, M.-y., Yang, H.-j., Xu, X.-l., & Zhou, G.-h. (2018). The effect of pressure-assisted heating on the water holding capacity of chicken batters. *Innovative food science & emerging technologies, 45*, 280-286. doi: 10.1016/j.ifset.2017.11.011

- Zhu, X., Zhang, Z., Hinds, L. M., Sun, D.-W., & Tiwari, B. K. (2021). Applications of ultrasound to enhance fluidized bed drying of Ascophyllum Nodosum: Drying kinetics and product quality assessment. *Ultrasonics sonochemistry, 70*, 105298-105298. doi: 10.1016/j.ultsonch.2020.105298

- Zhu, Y., Yan, Y., Yu, Z., Wu, T., & Bennett, L. E. (2022). Effects of high pressure processing on microbial, textural and sensory properties of low-salt emulsified beef sausage. *Food control, 133*, 108596. doi: 10.1016/j.foodcont.2021.108596

- Zhuang, X., Han, M., Kang, Z.-l., Wang, K., Bai, Y., Xu, X.-l., & Zhou, G.-h. (2016). Effects of the sugarcane dietary fiber and pre-emulsified sesame oil on low-fat meat batter physicochemical property, texture, and microstructure. *Meat Science, 113*, 107-115. doi: 10.1016/j.meatsci.2015.11.007

Appendices

Appendix i: Survey Questionnaire used for chapter 2

Information sheet

Thank you for considering participating in this research project. The purpose of this document is to explain to you what the work is about and what participation would involve, enabling you to make an informed decision on whether or not to participate.

This study examines consumer reactions to the reduction/removal of phosphate additives that are currently added to processed meat products such as sausages and burgers. Should you choose to participate, you will be asked to complete a questionnaire, which will include questions on socio-economic factors such as age, income, and education, as well as questions concerning your attitudes and behaviours relating to processed meat products.

Participation in this study is completely voluntary. All the information that you provide will be confidential, and your anonymity will be protected throughout the study.

The information you provide may contribute to research publications, PhD thesis research report and/or conference presentations. Should you experience any distress arising from participating in the research or have any queries, you can contact Karthikeyan Palanisamy Thangavelu at Karthikeyan.palanisamythangavelu@teagasc.ie, Dr. Carlos Alvarez at Carlos.alvarez@teagasc.ie, Dr Brijesh Tiwari at Brijesh.Tiwari@teagasc.ie or Prof. Joe Kerry at Joe.kerry@ucc.ie

This study has obtained ethical approval from the UCC Social Research Ethics Committee.

If you agree to take part in this study, please complete the consent form below.

Research Consent Form

Do you consent to participate in this study?

Yes □ No - Close □

Exclusion Criteria

Have you been living in Ireland for more than 3 years?

Yes □ No - Close □

How often do you consume meat and meat products as a part of your diet?

- Daily basis
- 5-6 times per week
- 2-4 times per week
- Once a week
- 1-3 times per month
- Less than once per month - Close
- Never - Close

Do you work (or have you previously worked) in the area of food regulation?

Yes - close □ No □

Do you work (or have you previously worked) in the area of food science & technology?

Yes – close □ No □

Thank you for your time and participation. Unfortunately, it appears that you do not fit the profile required for this survey.

Questionnaire

Section 1: Sociodemographic characteristics

1. Are you?
    a. Male
    b. Female
    c. Prefer not to say
2. Which of the following age groups are you in?
    a. 18-24 years
    b. 25-34 years

   c. 35-44 years
   d. 45-54 years
   e. 55-64 years
   f. 65 years and above

3. How would you describe where you live?
   a. Urban City
   b. Urban town
   c. Suburban
   d. Rural village
   e. Rural Countryside
4. What is the highest level of education that you have completed?
   a. No formal education
   b. Primary
   c. Secondary
   d. Post-secondary (non-tertiary, e.g. certificate or diploma)
   e. Undergraduate tertiary degree (i.e. Bachelor's degree)
   f. Postgraduate degree (i.e. Masters, PhD)
   g. Others (please specify)
   h. Prefer not to say
5. What is your current employment status? If more than one option is applicable, e.g., you are employed part-time and a student, please select the activity that takes up the most of your time.
   a. Working full time (35 or more hours per week)
   b. Working part-time (less than 35 hours per week)
   c. Self employed
   d. Unemployed and looking for work
   e. Looking after my home/family full time
   f. Student
   g. Retired
   h. Unable to work
   i. Other, please specify

6. Please indicate to which occupational group the chief income earner in your household belongs, or which groups fits best. The chief income earner is the person in your household with the largest income; this could be yourself or another person in your household.
    a. Higher Managerial/Professional/Administrative (i.e., Established Doctor; Dentist; Psychologist; Solicitor; Board Director)
    b. Intermediate Managerial/Professional/Administrative (i.e., Newly qualified (under 3 years) Doctor; Solicitor; Board Director)
    c. Supervisory/Clerical/ Junior Managerial or Professional or Administrative (i.e., Office worker; Student Doctor; Teacher; Nurse)
    d. Student
    e. Skilled manual worker (Pub or Bar worker; Skilled Bricklayer; Carpenter; Plumber; Electrician; Painter; Bus or Ambulance)
    f. Semi or unskilled manual worker (Manual worker; Apprentice to the skilled trade; Labourer; Cleaner; Shop assistant; Waiter)
    g. Casual worker – not in permanent employment
    h. Homemaker/In-Home duties
    i. Retired
    j. Farmer 1-49 acres
    k. Farmer 50+ acres
    l. Unemployed or not working due to long-term sickness
7. In which of the following bands would you place your household's combined yearly income (before tax and other deductions)? Household income is a measure of the combined income of all the people sharing a particular household or place of residence.
    a. Less than €30,000
    b. €30,000 - €59,999
    c. €60,000 - €89,999
    d. €90,000 - €119,999
    e. €120,000 - €149,999
    f. €150,000 or above

Section 2 – Processed meat products and phosphate additives

"Processed meat products" is a term used in food research to refer to meat that has been cooked, smoked, cured or changed in some manner. Phosphates are food additives used in processed meat foods as stabilisers, thickeners and emulsifiers. They are sometimes expressed as E – numbered (E 388 – 342) additives in the meat product label.

1. How often do you consume the following processed meat products?

	Daily or more often	5-6 times per week	2-4 times per week	Only once per week	1-3 times per month	Less than once per month	Never
Sausages							
Burgers							
Hot dogs							
Pastrami							
Bacon							
Smoked or cured ham slices							
Pre-cooked Roast beef slices							
Pre-cooked Corned Beef slices							

2. Why do you think food companies use food additives in processed meat products such as sausages, burgers, hams? (Tick all that apply)

    a. To improve the shelf-life of the products
    b. To improve the flavour and colour of the products
    c. To mprove the stability and appearance of the products
    d. To increae the weight of the products
    e. To improve the eating quality of the products
    f. To mask any adverse qulaity aspects of the raw materials used
    g. To treat, cure or prevent certain deficiency or diseases such as cancer
    h. Others (please specify)
    i. Don't know

3. How would you rate your level of knowledge of food addditives in processed meat products on a 7-point scale?

1	2	3	4	5	6	7
Extremely Poor	Poor	Satisfactory	Moderate	Good	Excellent	Outstanding

Below you will see some statements others have made about phosphate additives, please indicate how much you agree or disagree with each of the statements on a scale of 1 -7 where 1 being strongly disagree and 7 being strongly agree.

1. Phosphates are added to improve the juiciness, flavour, and/or eating quality of processed meat products

1	2	3	4	5	6	7
Strongly disagree	Disagree	Somewhat disagree	Neutral	Somewhat agree	Agree	Strongly agree

2. Excess consumption of processed meats containing added phosphates leads to serious health issues in people

1	2	3	4	5	6	7
Strongly disagree	Disagree	Somewhat disagree	Neutral	Somewhat agree	Agree	Strongly agree

3. Reduction of phosphates in processed meat products such as burgers and sausages can make them dry and unpalatable

1	2	3	4	5	6	7
Strongly disagree	Disagree	Somewhat disagree	Neutral	Somewhat agree	Agree	Strongly agree

4. Reduction of phosphates in processed meat products for health benefits is acceptable even when the eating quality of the product is reduced

1	2	3	4	5	6	7
Strongly disagree	Disagree	Somewhat disagree	Neutral	Somewhat agree	Agree	Strongly agree

5. How important, if at all, do you think it is important or unimportant using the scale provided, where 1 is Very unimportant and 7 is very important. Please note

that there are no right or wrong answers in this question, only your opinion. Reduction of addedd phosphates in processed meat products.

1	2	3	4	5	6	7
Very unimportant	Unimportant	Somewhat unimportant	Neutral	Somewhat important	Important	Very important

Section 3 – Theory of Planned Behaviour constructs

1. Attitudes

1. Consuming processed meat products containing reduced amount of added phosphates is

1	2	3	4	5	6	7
Highly unsatisfactory	Unsatisfactory	Somewhat unsatisfactory	Neutral	Somewhat satisfactory	Satisfactory	Highly satisfactory

2. Consuming processed meat products containing reduced amount of added phosphates is

1	2	3	4	5	6	7
Very unimportant	Unimportant	Somewhat unimportant	Neutral	Somewhat important	Important	Very important

3. Consuming processed meat products containing reduced amount of added phosphates is

1	2	3	4	5	6	7
Highly disadvantageous	Disadvantageous	Somewhat disadvantageous	Neutral	Somewhat advantageous	Advantageous	Highly advantageous

4. Consuming processed meat products containing reduced amount of added phosphates is

1	2	3	4	5	6	7
Very unacceptable	Unacceptable	Somewhat unacceptable	Neutral	Somewhat acceptable	Acceptable	Very acceptable

2. Subjective Norms

1. Friends who influence my purchase decisions think I should buy processed meat products with reduced levels of added phosphates

1	2	3	4	5	6	7
Strongly disagree	Disagree	Somewhat disagree	Neutral	Somewhat agree	Agree	Strongly agree

2. Family members who influence my purchase decisions think I should buy processed meat products with reduced levels of added phosphates

1	2	3	4	5	6	7
Strongly disagree	Disagree	Somewhat disagree	Neutral	Somewhat agree	Agree	Strongly agree

3. I feel I am under social pressure to buy processed meat products with reduced level of added phosphates

1	2	3	4	5	6	7
Strongly disagree	Disagree	Somewhat disagree	Neutral	Somewhat agree	Agree	Strongly agree

3. Perceived Behavioural Control

1. Processed meat products with reduced levels of added phosphates are not widely available

1	2	3	4	5	6	7
Strongly disagree	Disagree	Somewhat disagree	Neutral	Somewhat agree	Agree	Strongly agree

2. I do not know how to distinguish processed meat products with reduced levels of added phosphates from conventional ones in the supermarkets, butchers or other outlets

1	2	3	4	5	6	7
Strongly disagree	Disagree	Somewhat disagree	Neutral	Somewhat agree	Agree	Strongly agree

3. I am confident that I could identify processed meat products that have reduced levels of added phosphates in the supermarket, butchers or other outlets

1	2	3	4	5	6	7
Strongly disagree	Disagree	Somewhat disagree	Neutral	Somewhat agree	Agree	Strongly agree

4. Perceived Health Risks

1. I believe that the regular consumption of processed meat products with added phosphates is unhealthy

1	2	3	4	5	6	7
Strongly disagree	Disagree	Somewhat disagree	Neutral	Somewhat agree	Agree	Strongly agree

2. It is not a safe strategy by the meat industry to use added phosphates in processed meat products

1	2	3	4	5	6	7
Strongly disagree	Disagree	Somewhat disagree	Neutral	Somewhat agree	Agree	Strongly agree

3. I think that processed meat products containing reduced levels of added phosphates are safer to eat

1	2	3	4	5	6	7
Strongly disagree	Disagree	Somewhat disagree	Neutral	Somewhat agree	Agree	Strongly agree

5. Intention

1. I expect to purchase processed meat products with reduced levels of added phosphates in the next month

1	2	3	4	5	6	7
Strongly disagree	Disagree	Somewhat disagree	Neutral	Somewhat agree	Agree	Strongly agree

2. I want to purchase processed meat products with reduced levels of added phosphates in the next month

1	2	3	4	5	6	7
Strongly disagree	Disagree	Somewhat disagree	Neutral	Somewhat agree	Agree	Strongly agree

3. I intend to purchase processed meat products with reduced levels of added phosphates in the next month

1	2	3	4	5	6	7
Strongly disagree	Disagree	Somewhat disagree	Neutral	Somewhat agree	Agree	Strongly agree

Thank you for your participation in the survey.